US009557217B2

(12) **United States Patent**
Zimenkov et al.

(10) **Patent No.: US 9,557,217 B2**
(45) **Date of Patent: Jan. 31, 2017**

(54) **UNIVERSAL MULTIDETECTION SYSTEM FOR MICROPLATES**

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(73) Assignee: **BTI HOLDINGS, INC.**, Winooski, VT (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 333 days.

(21) Appl. No.: **13/485,446**

(22) Filed: **May 31, 2012**

(65) **Prior Publication Data**

US 2012/0300194 A1 Nov. 29, 2012

Related U.S. Application Data

(60) Continuation-in-part of application No. 12/838,804, filed on Jul. 19, 2010, now Pat. No. 8,218,141, which

(Continued)

(51) **Int. Cl.**

G01J 3/42 (2006.01)
G01J 3/02 (2006.01)

(Continued)

(52) **U.S. Cl.**

CPC ***G01J 3/42*** (2013.01); ***G01J 3/027*** (2013.01); ***G01J 3/0218*** (2013.01); ***G01J 3/0224*** (2013.01);

(Continued)

(58) **Field of Classification Search**

CPC G01J 3/0224; G01J 3/0294; G01J 3/10; G01J 3/42; G01J 3/4406; G01J 3/443; G01J 3/0218; G01J 3/0235; G01J 3/0264; G01J 3/027; G01J 3/0291; G01J 3/28; G01J 3/2803; G01J 3/2823; G01J 2003/2806; G01J 2003/2813; G01J 2003/2816; G01J 2003/282; G01J 2003/2826; G01N 2021/6463; G01N 2021/6417; G01N 2021/6419; G01N 2021/6421; G01N 21/6456; G01N 21/645; G01N 21/763; G01N 21/64; G01N 21/6447; G01N 21/76; G01N 21/6452

See application file for complete search history.

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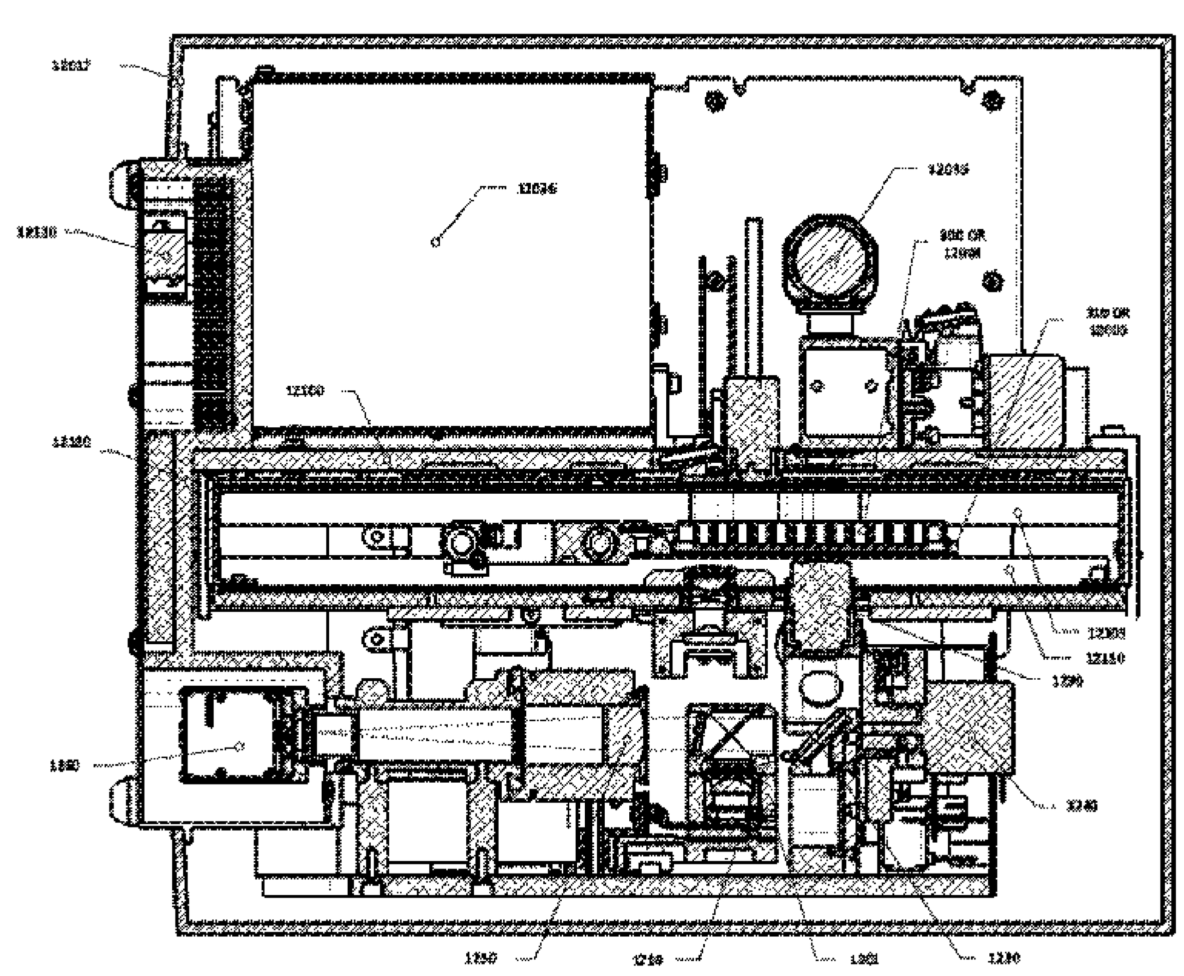
Primary Examiner — Gordon J Stock, Jr.

(74) *Attorney, Agent, or Firm* — Sughrue Mion, PLLC

(57) **ABSTRACT**

An apparatus for optically analyzing a sample may include an imaging subsystem that images the sample, one or more



analyzing subsystems that analyze the sample, a temperature control subsystem that controls a temperature of the atmosphere within the apparatus, a gas control subsystem that controls a composition of the atmosphere within the apparatus, and a control module that controls the various subsystems of the apparatus.

18 Claims, 30 Drawing Sheets

Related U.S. Application Data

is a division of application No. 11/802,831, filed on May 25, 2007, now Pat. No. 7,782,454.

(60) Provisional application No. 60/900,976, filed on Feb. 13, 2007.

(51) **Int. Cl.**
G01J 3/443 (2006.01)
G01N 21/64 (2006.01)
G01N 21/76 (2006.01)
G01J 3/10 (2006.01)
G01J 3/44 (2006.01)

(52) **U.S. Cl.**
CPC ***G01J 3/0235*** (2013.01); ***G01J 3/0264*** (2013.01); ***G01J 3/0291*** (2013.01); ***G01J 3/0294*** (2013.01); ***G01J 3/10*** (2013.01); ***G01J 3/443*** (2013.01); ***G01J 3/4406*** (2013.01); ***G01N 21/6452*** (2013.01); ***G01N 21/76*** (2013.01); *G01N 2021/6463* (2013.01)

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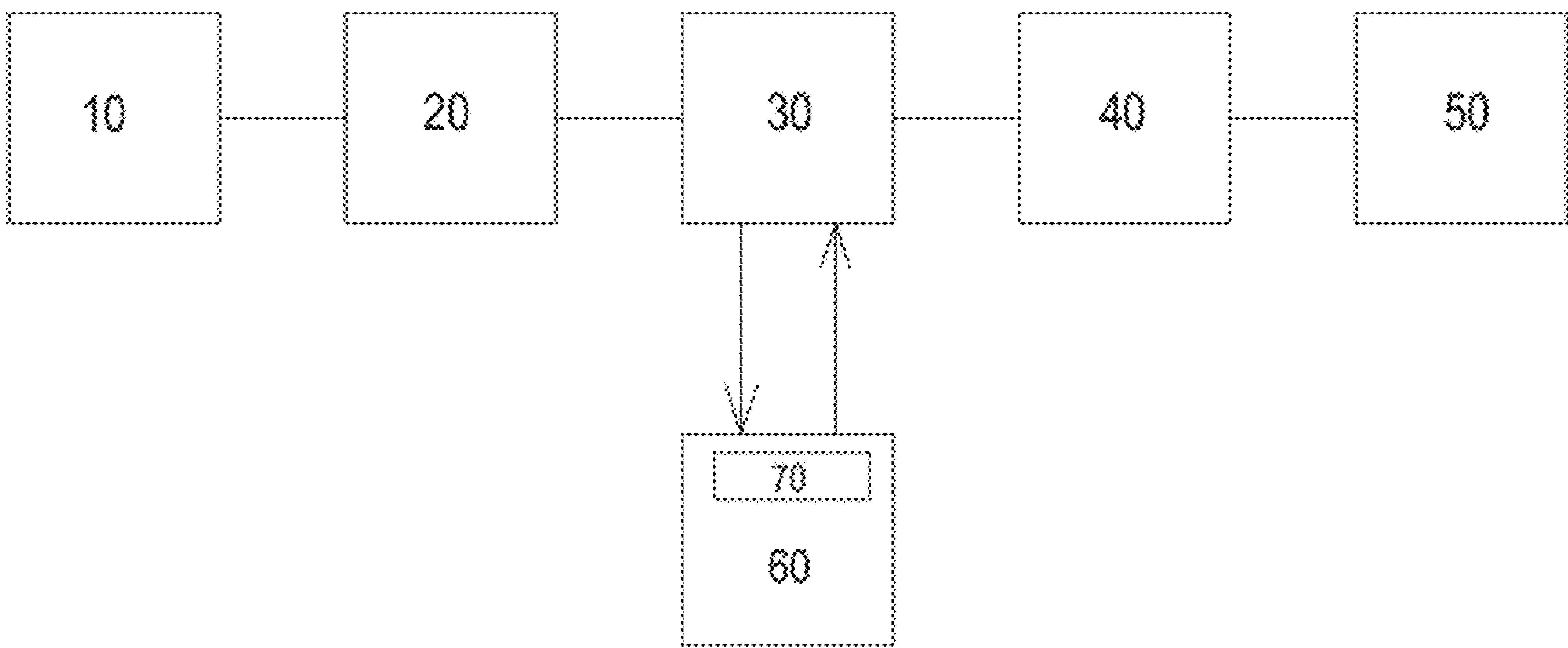
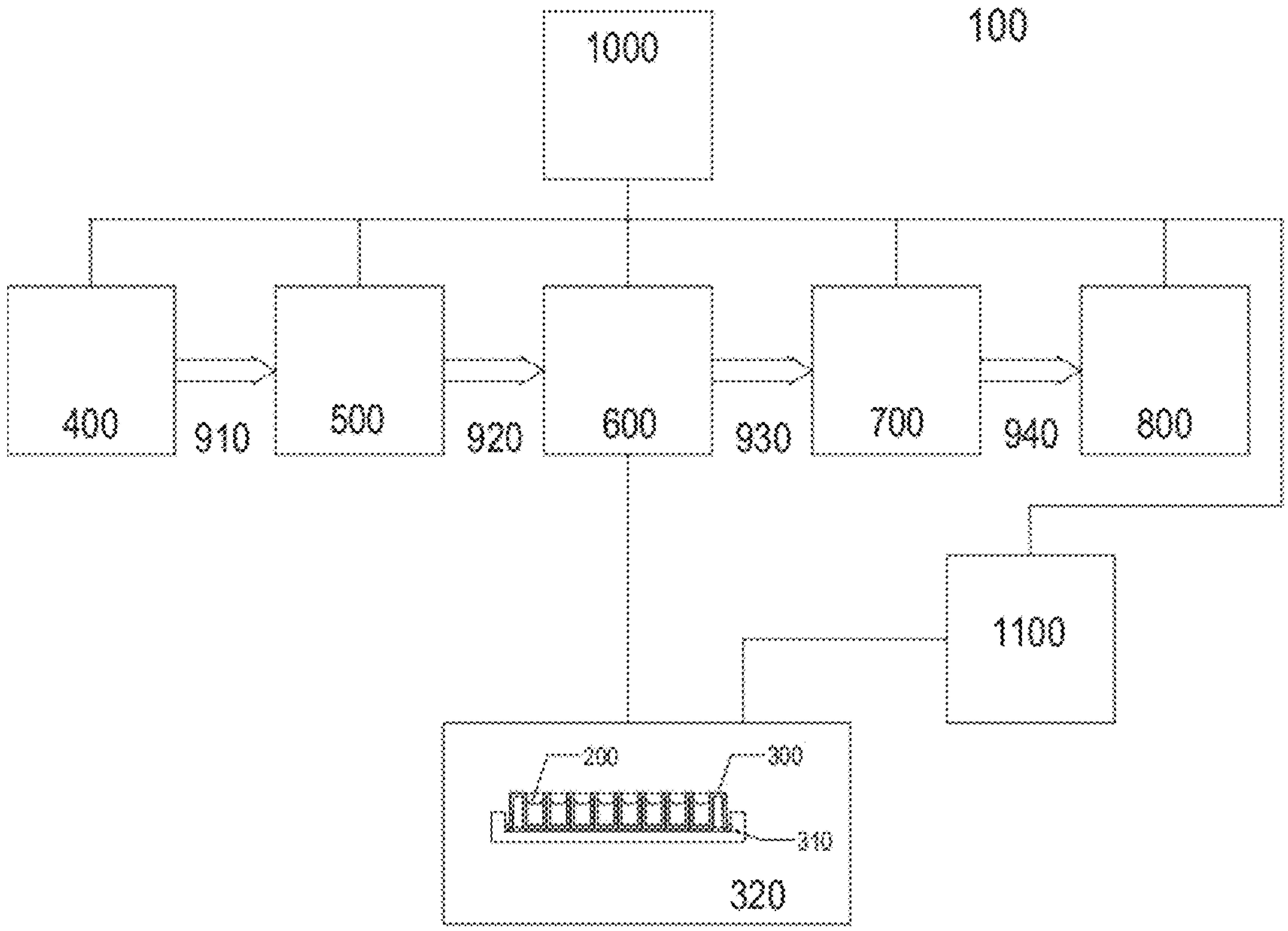


Fig. 1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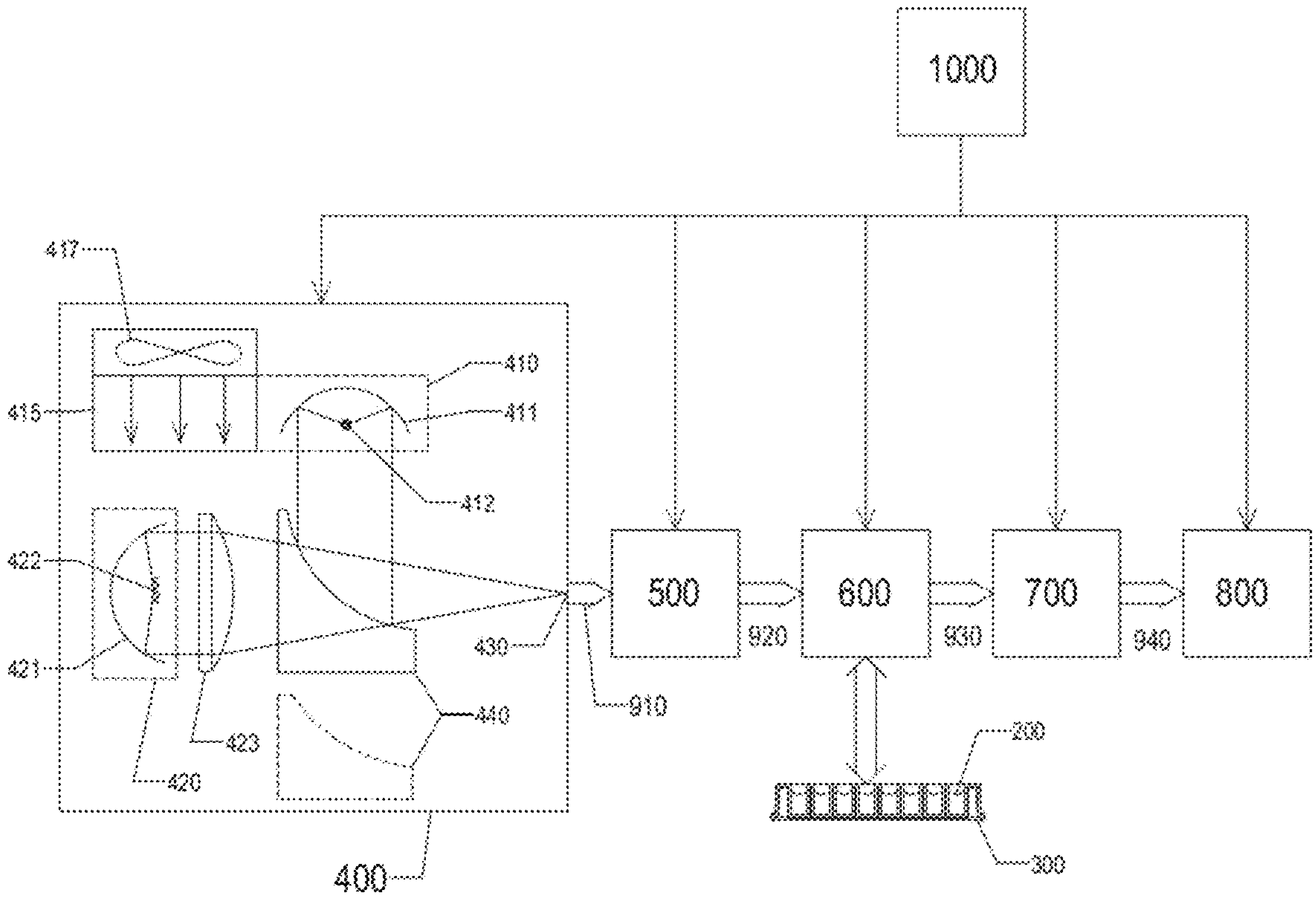
500
920
600
930
700
940
800
1100
200
300
310
320

Fig. 2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417
410
415
411
412
422
421
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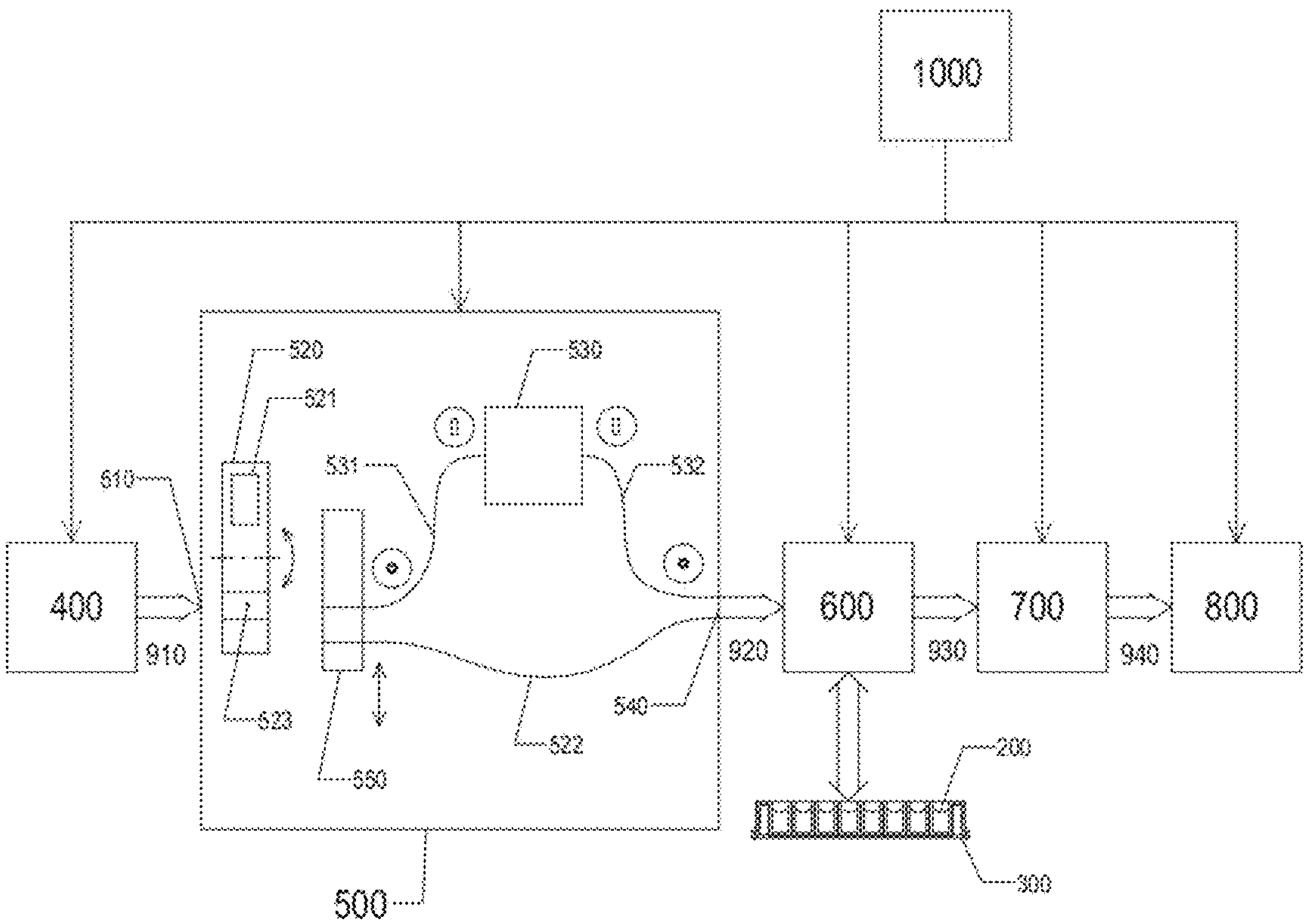
423
420
440
910
500
600
700
800
920
930
940
200
300
400

Fig. 3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400
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520
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523
530
531
532
540
550
510
522
910
920
930
940
200
300

Fig. 4

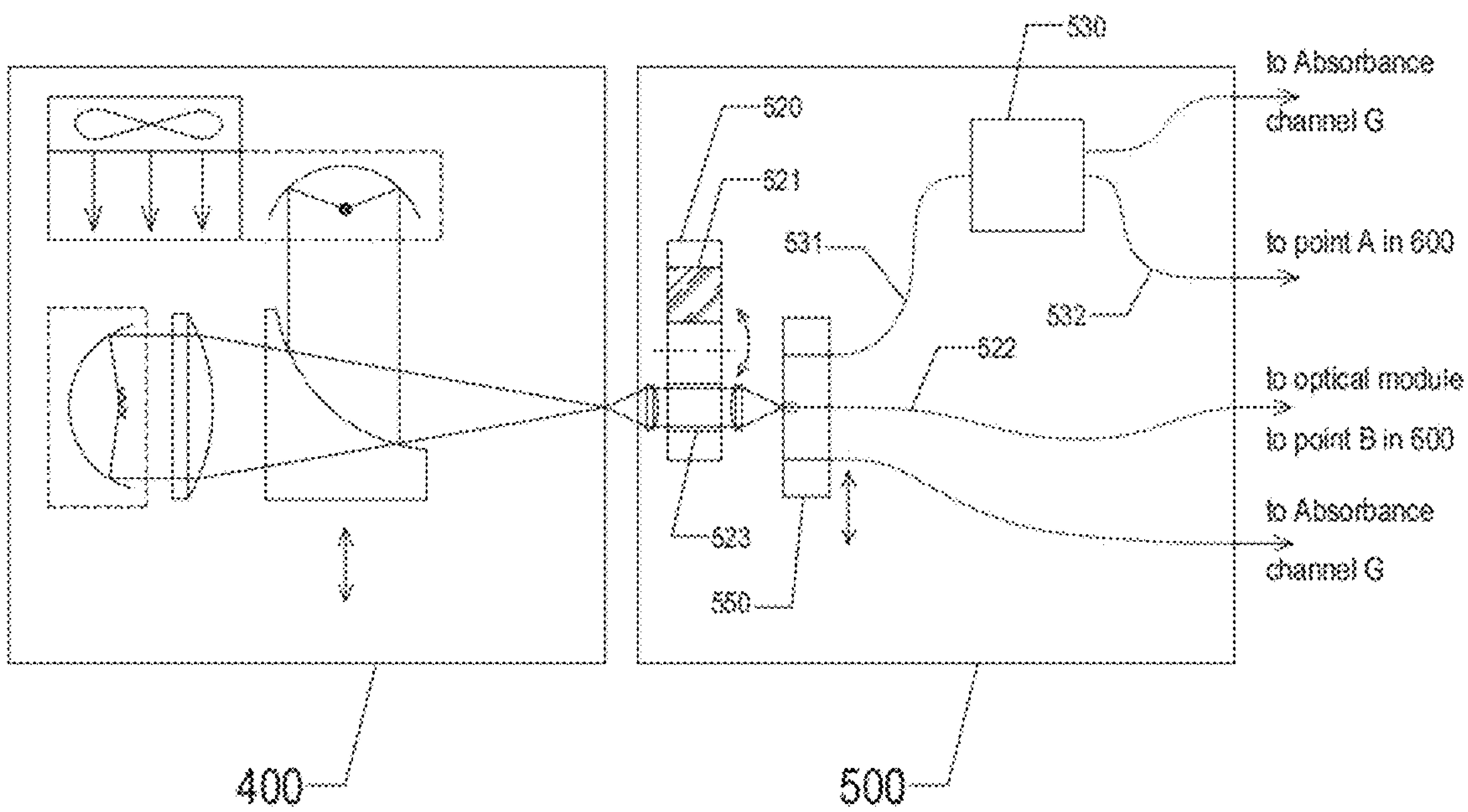

530
to Absorbance channel G
520
521
531
to point A in 600
532
522
to optical module
to point B in 600
523
to Absorbance channel G
550
400
500

Fig. 5

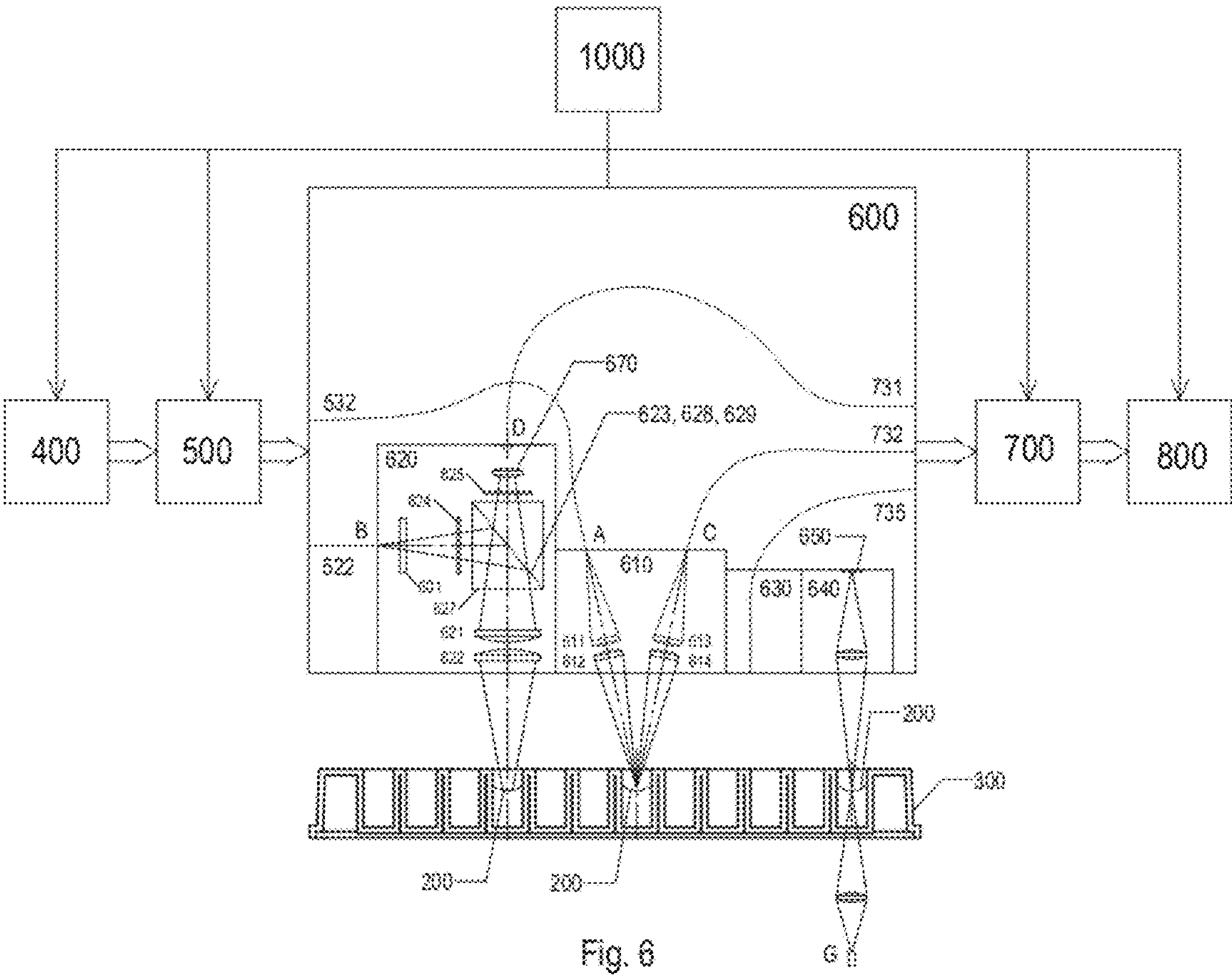

1000
600
400
500
700
800
670
623, 628, 629
532
731
732
735
D
620
625
624
B
A
C
522
610
650
630
640
621
622
611
612
613
614
200
300
200
200
G

Fig. 6

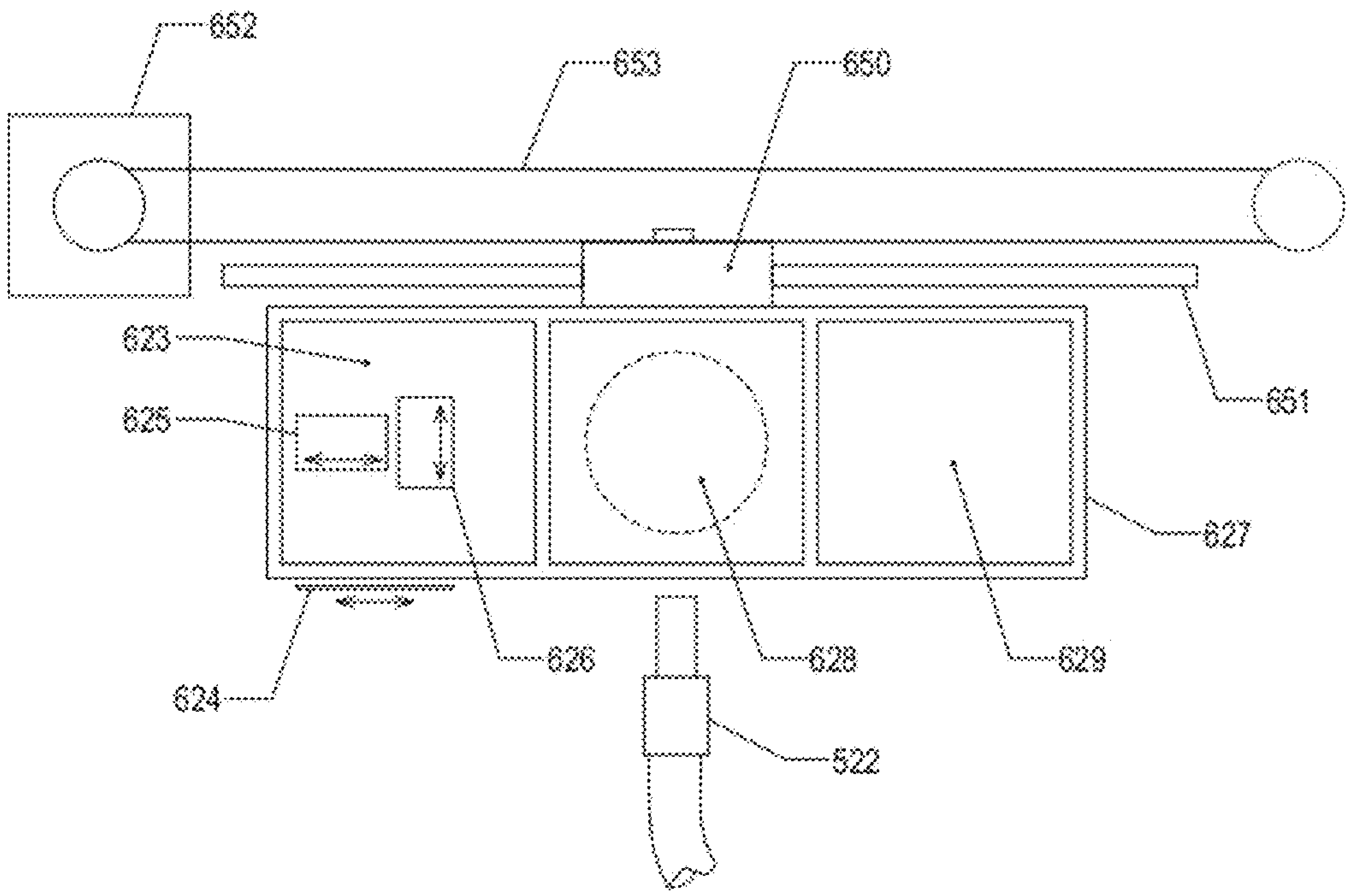


Fig. 7

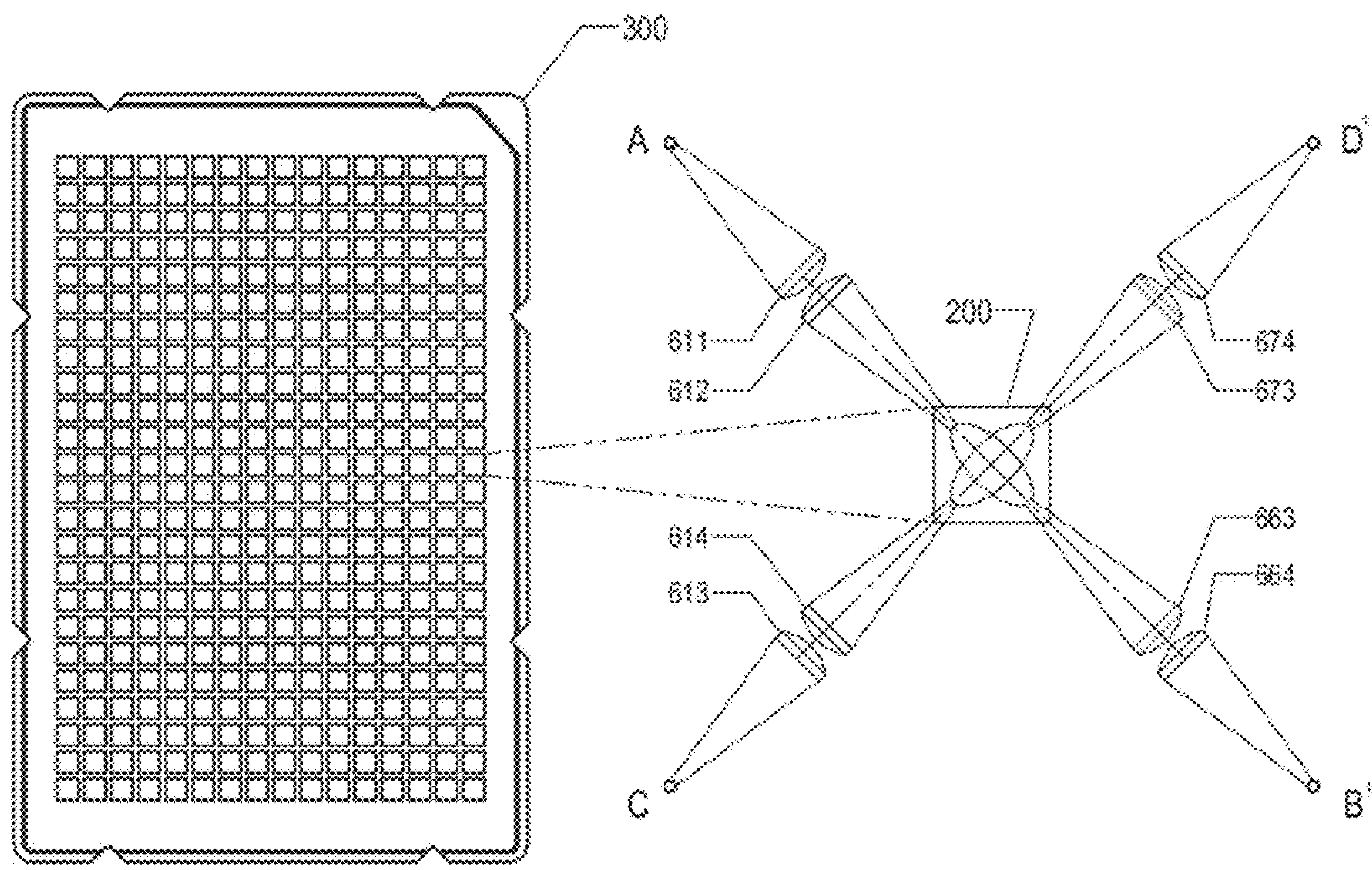


Fig. 8

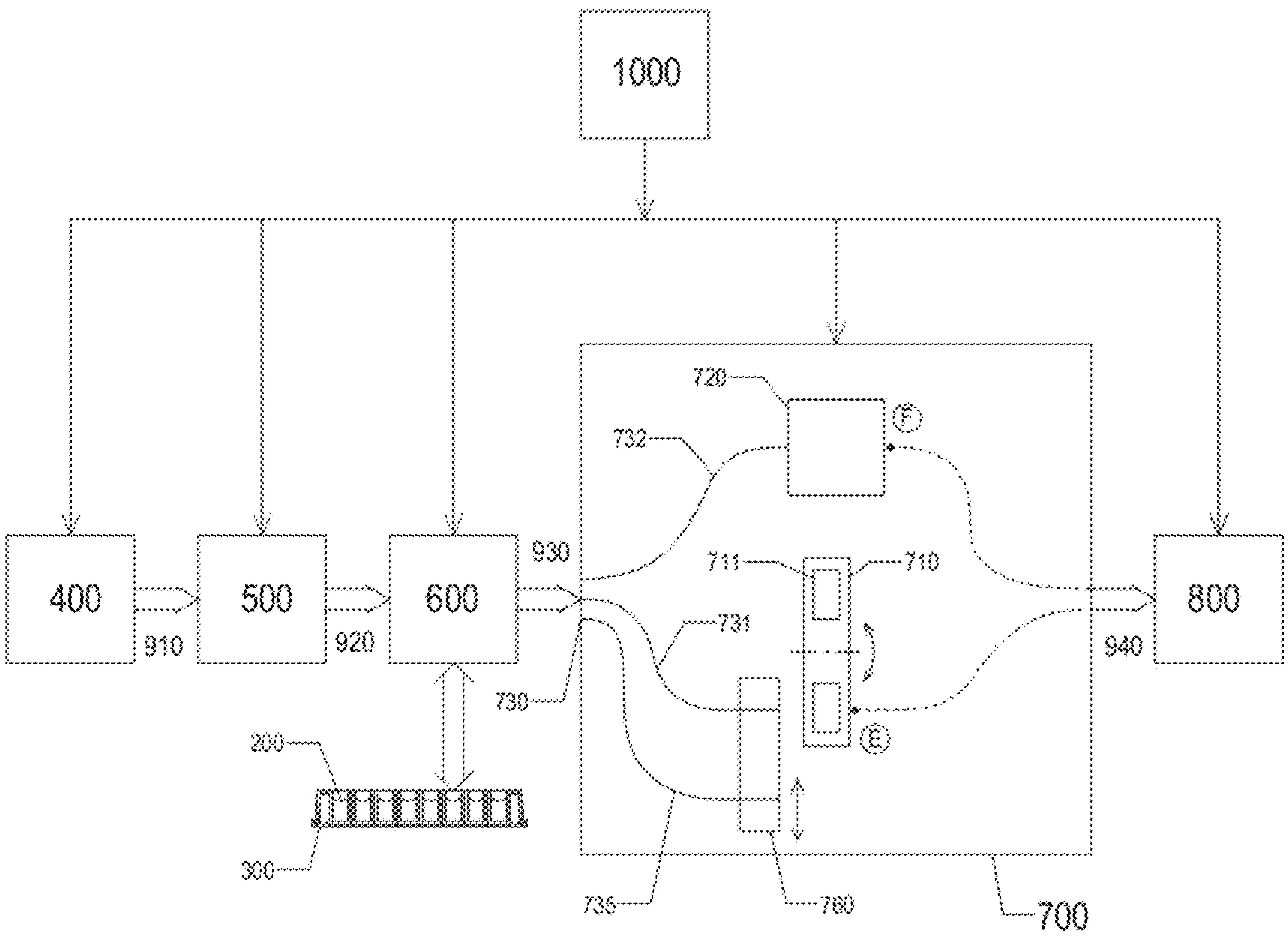

1000
400
500
600
800
910
920
930
940
720
732
711
710
731
730
200
300
735
760
700
F
E

Fig. 9

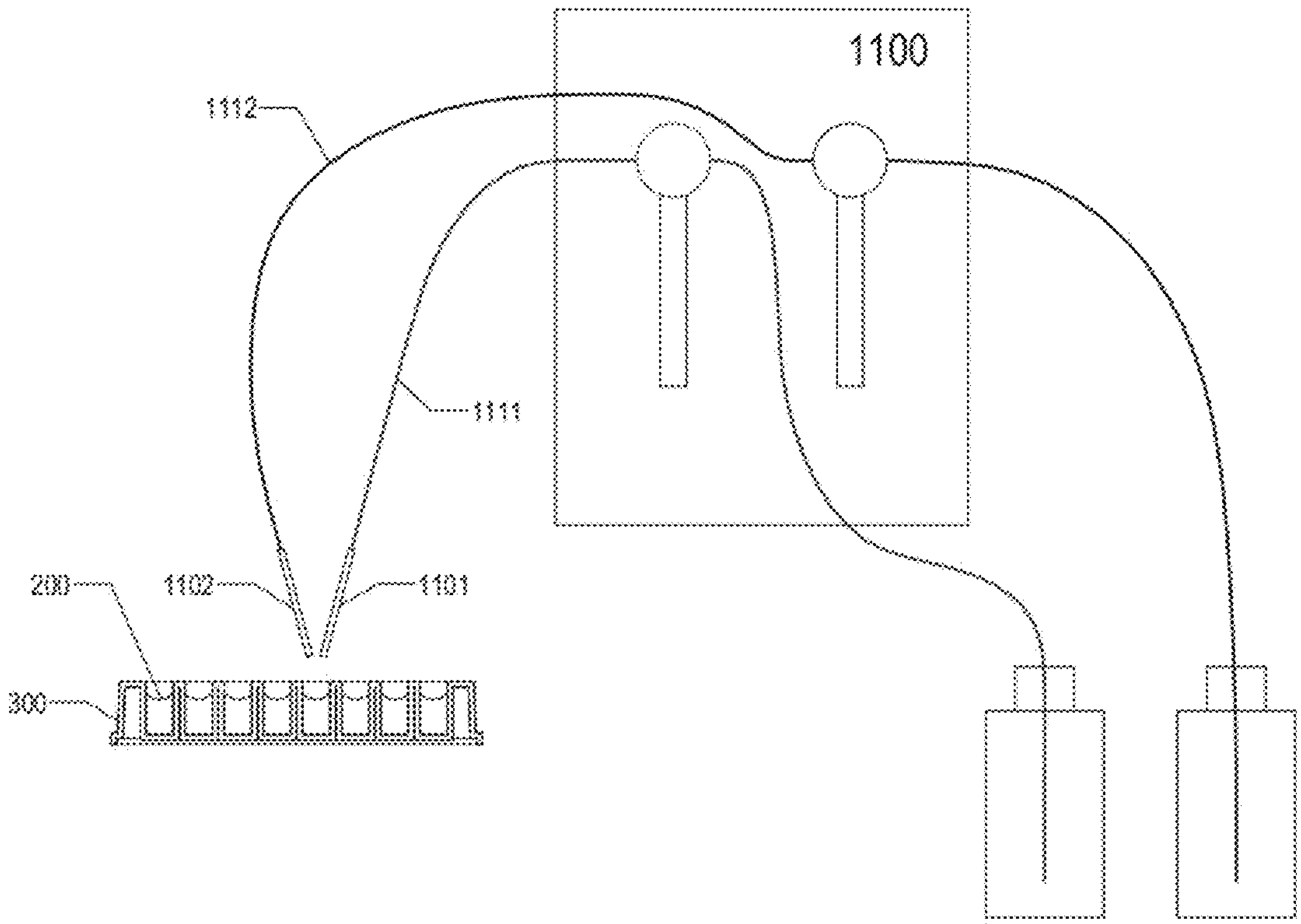


Fig. 10

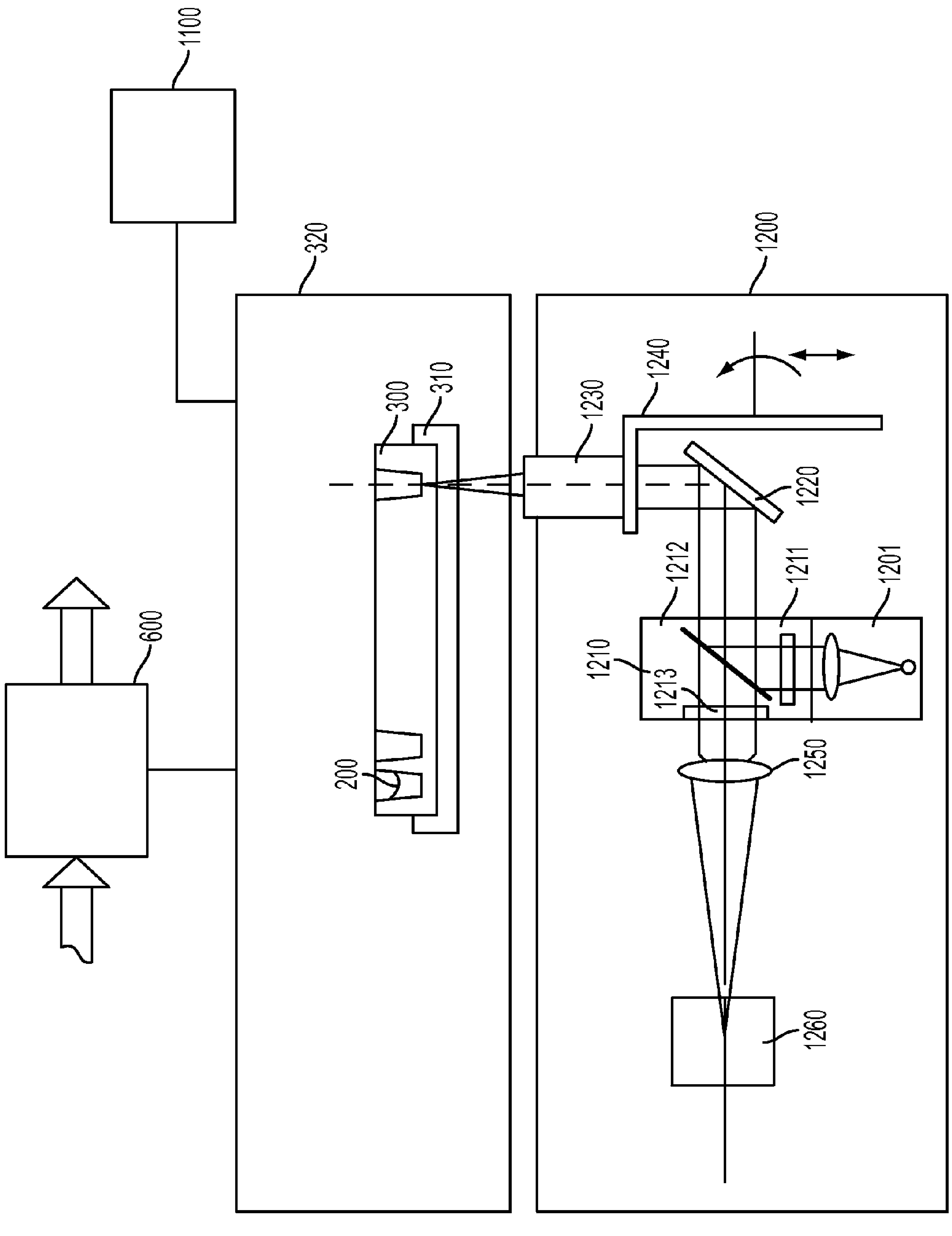

1100
320
1200
300
310
1230
1240
1220
1212
1211
1201
600
1210
1213
200
1250
1260

FIG. 11A

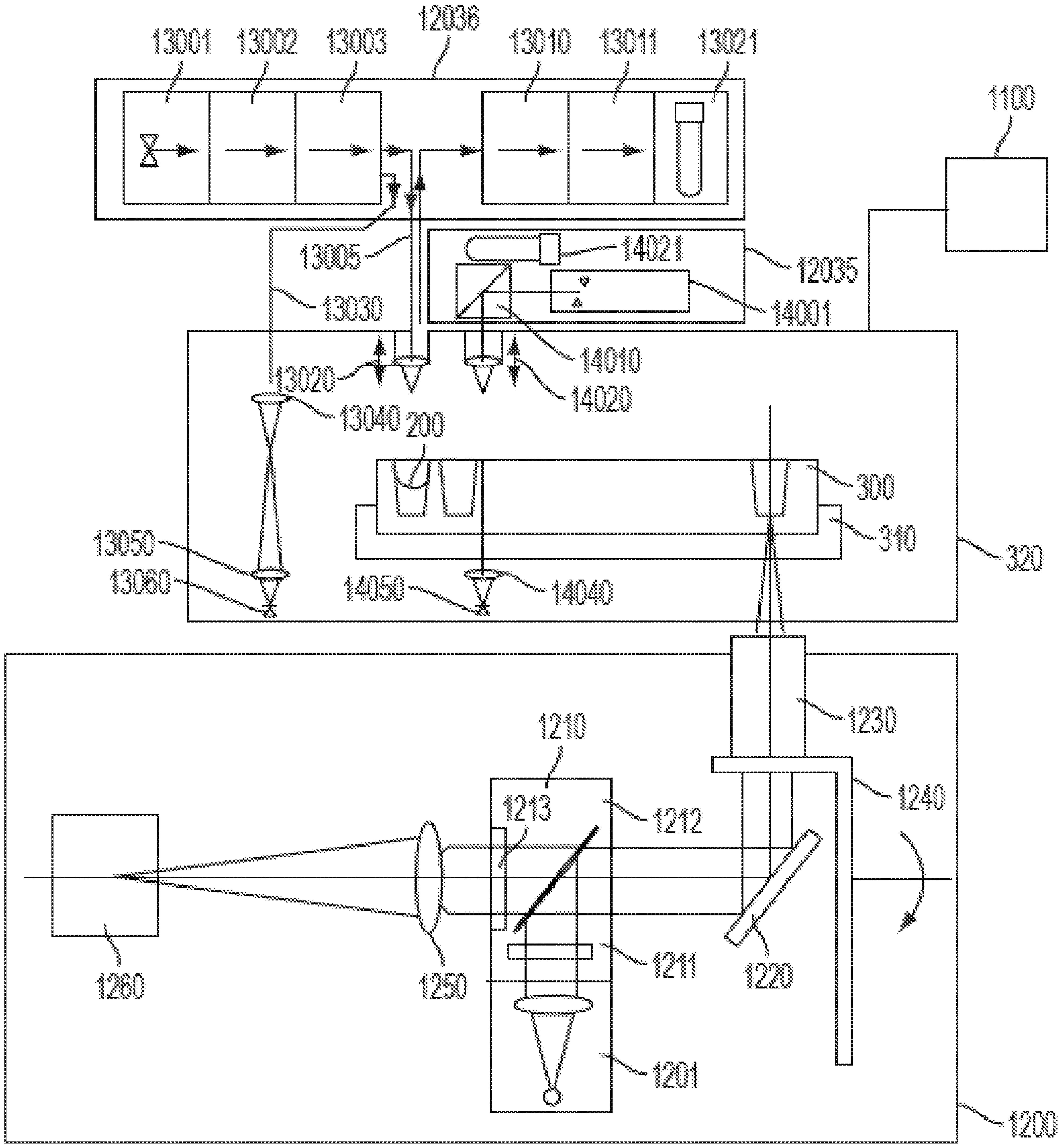

12036
13001
13002
13003
13010
13011
13021
1100
13005
14021
12035
13030
14001
14010
13020
14020
13040
200
300
310
320
13050
13060
14050
14040
1230
1210
1240
1213
1212
1211
1220
1260
1250
1201
1200

FIG. 11B

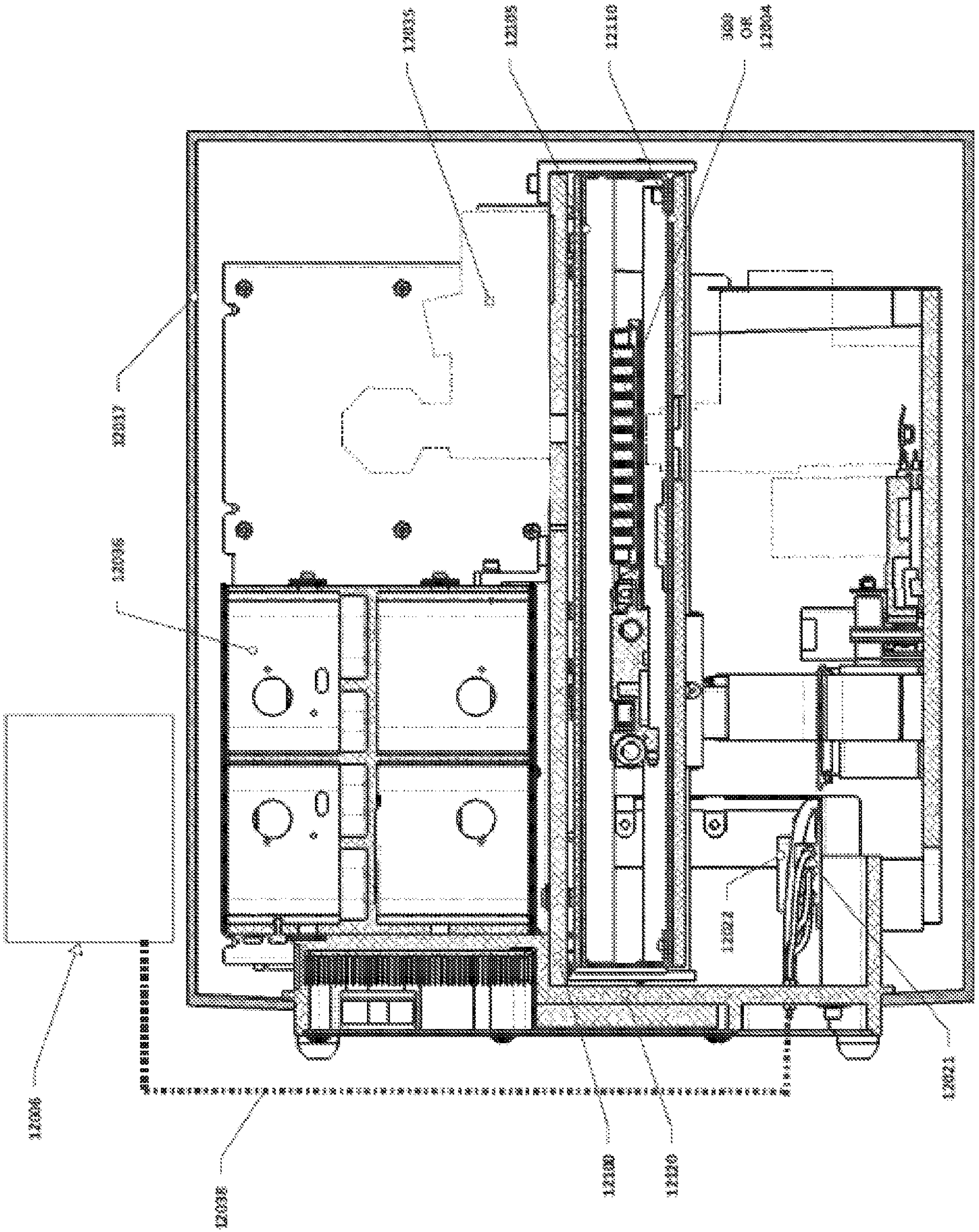


FIG. 12

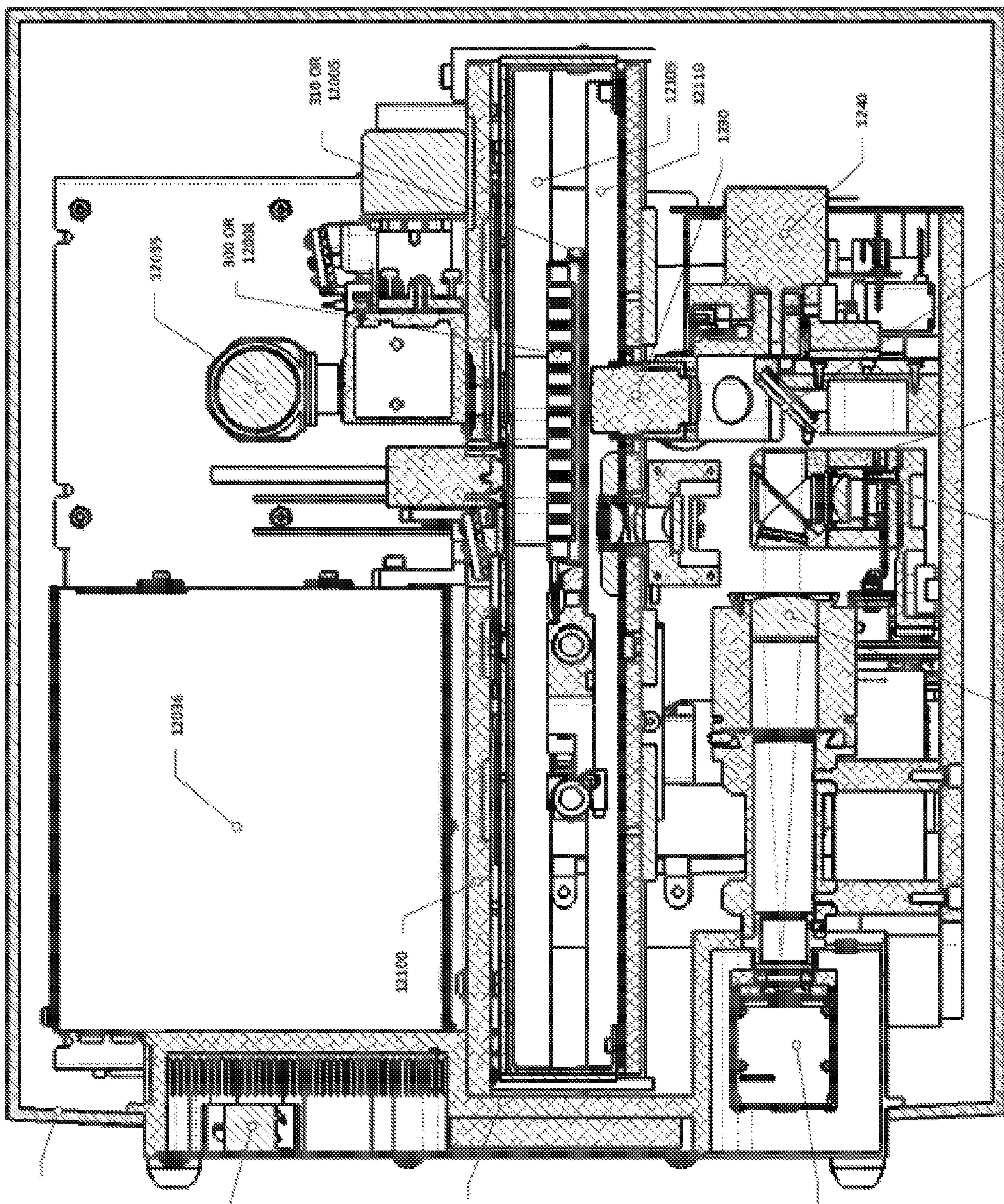


FIG. 13

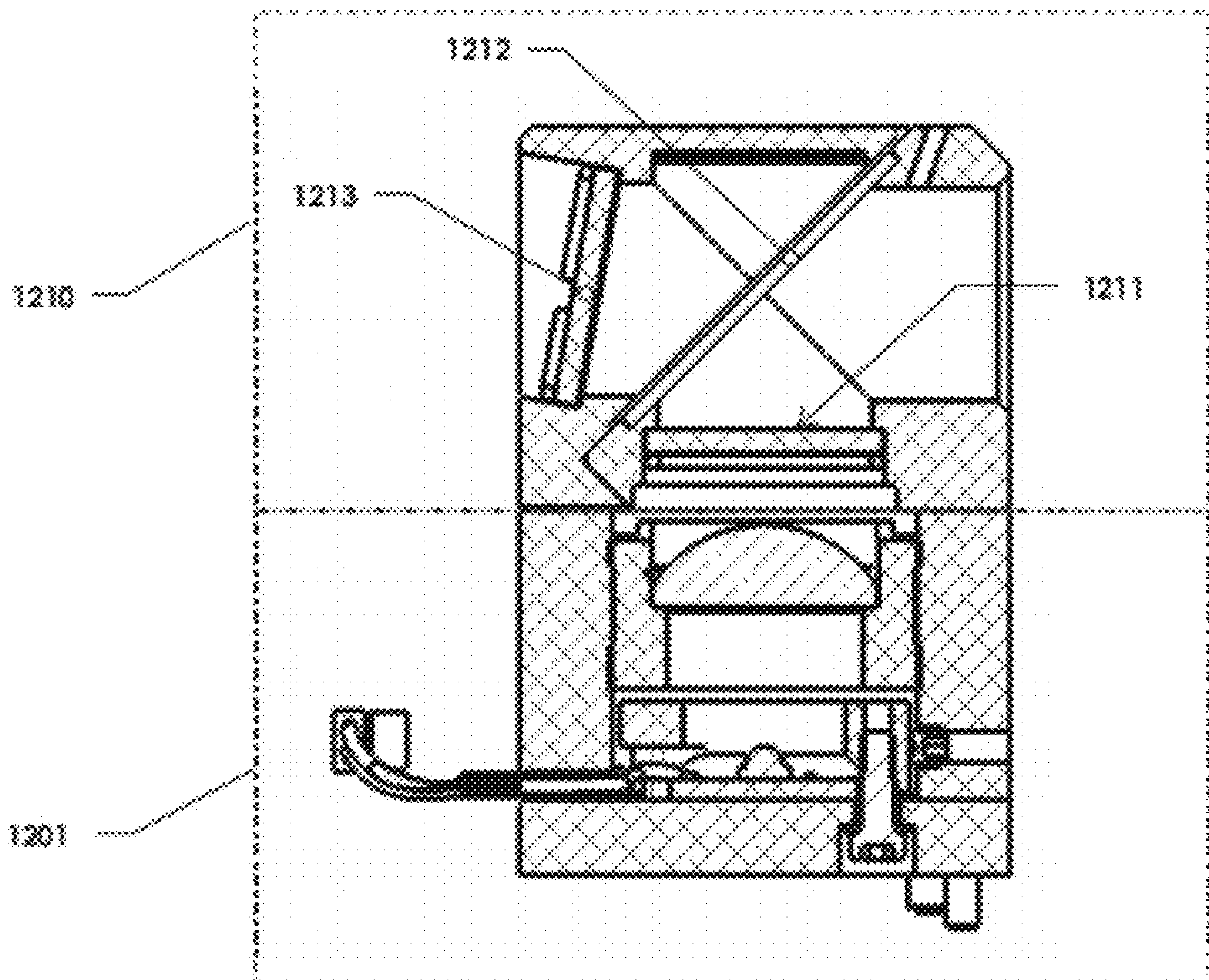


FIG. 14

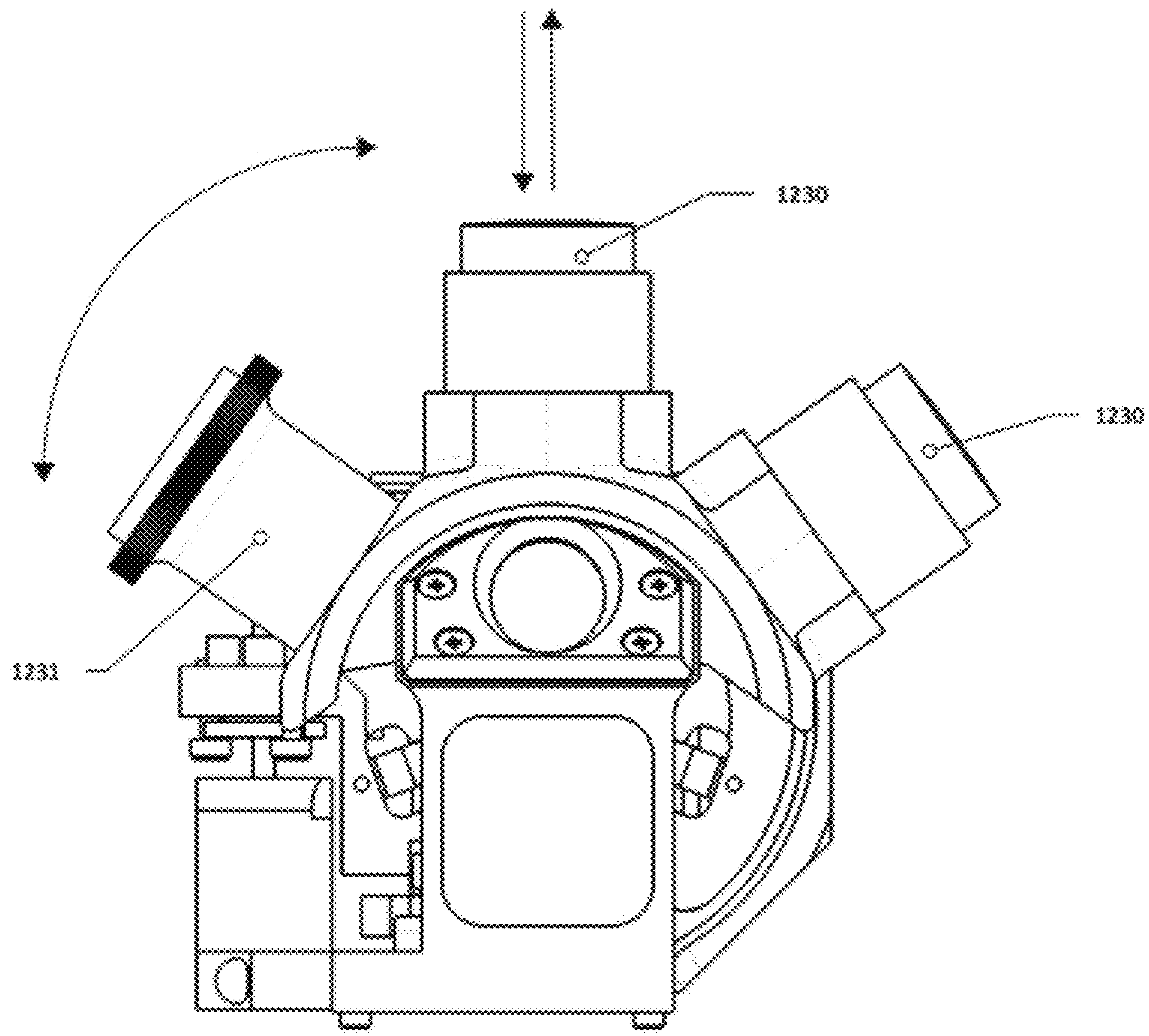


FIG. 15

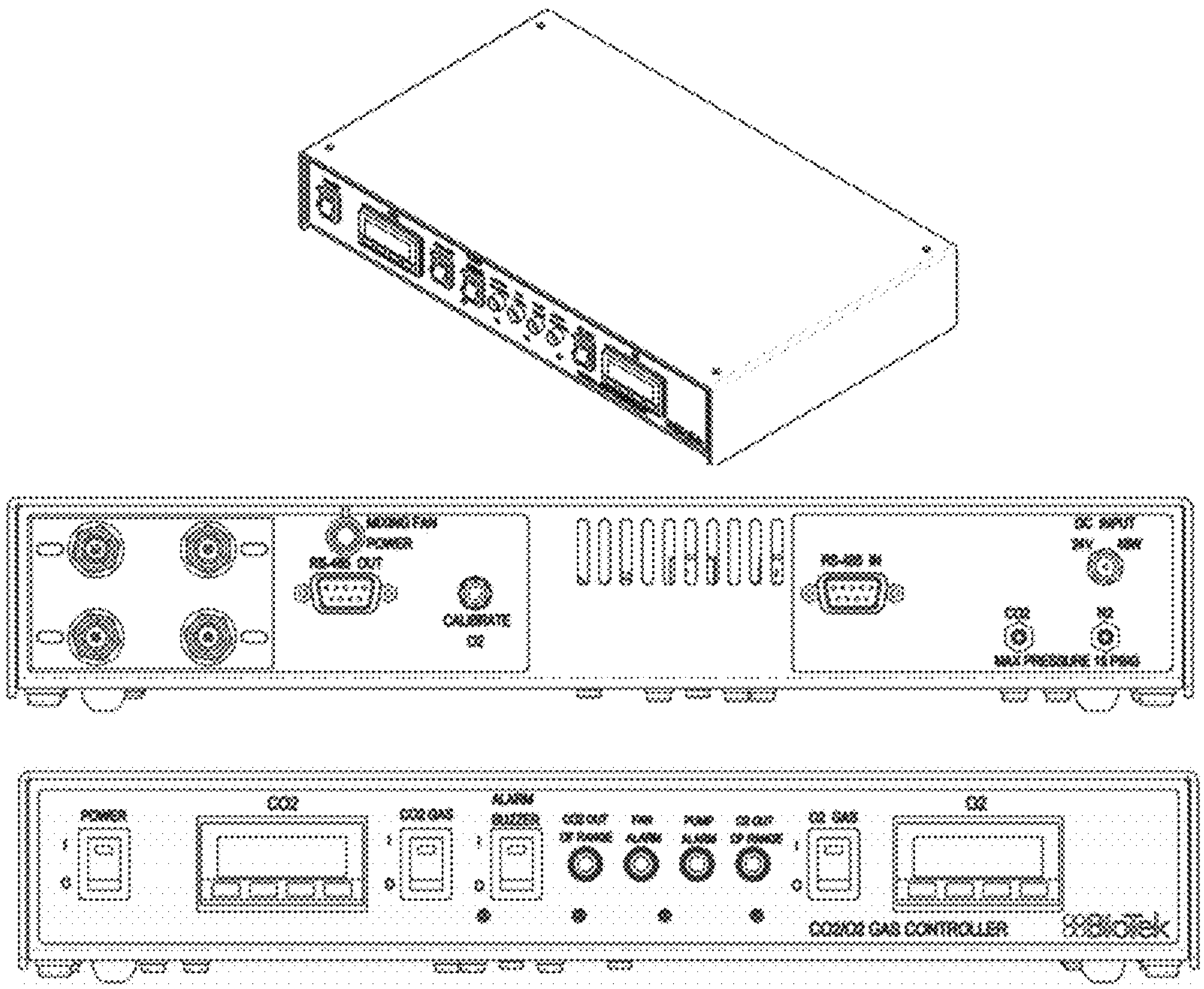


FIG. 16

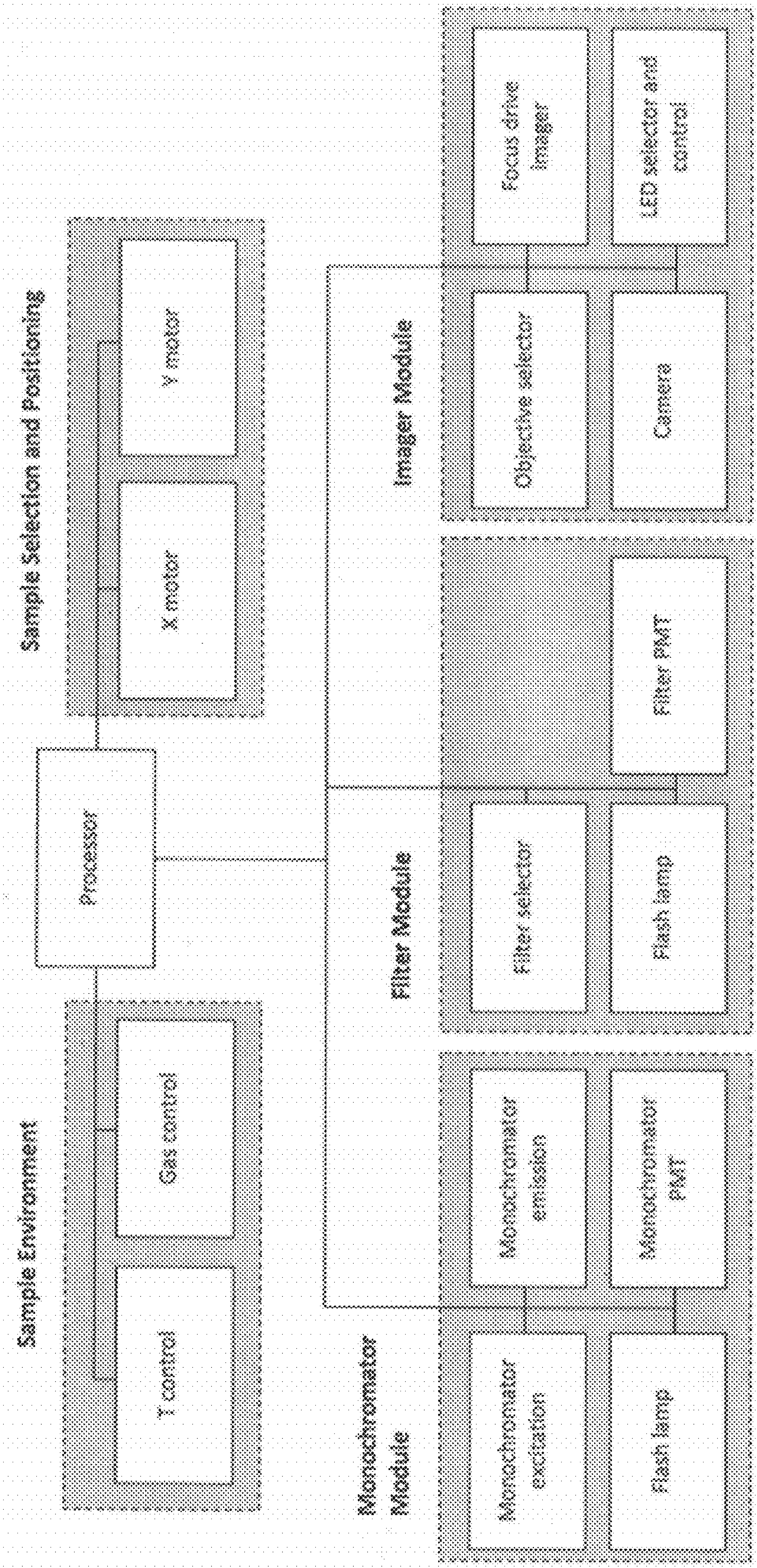
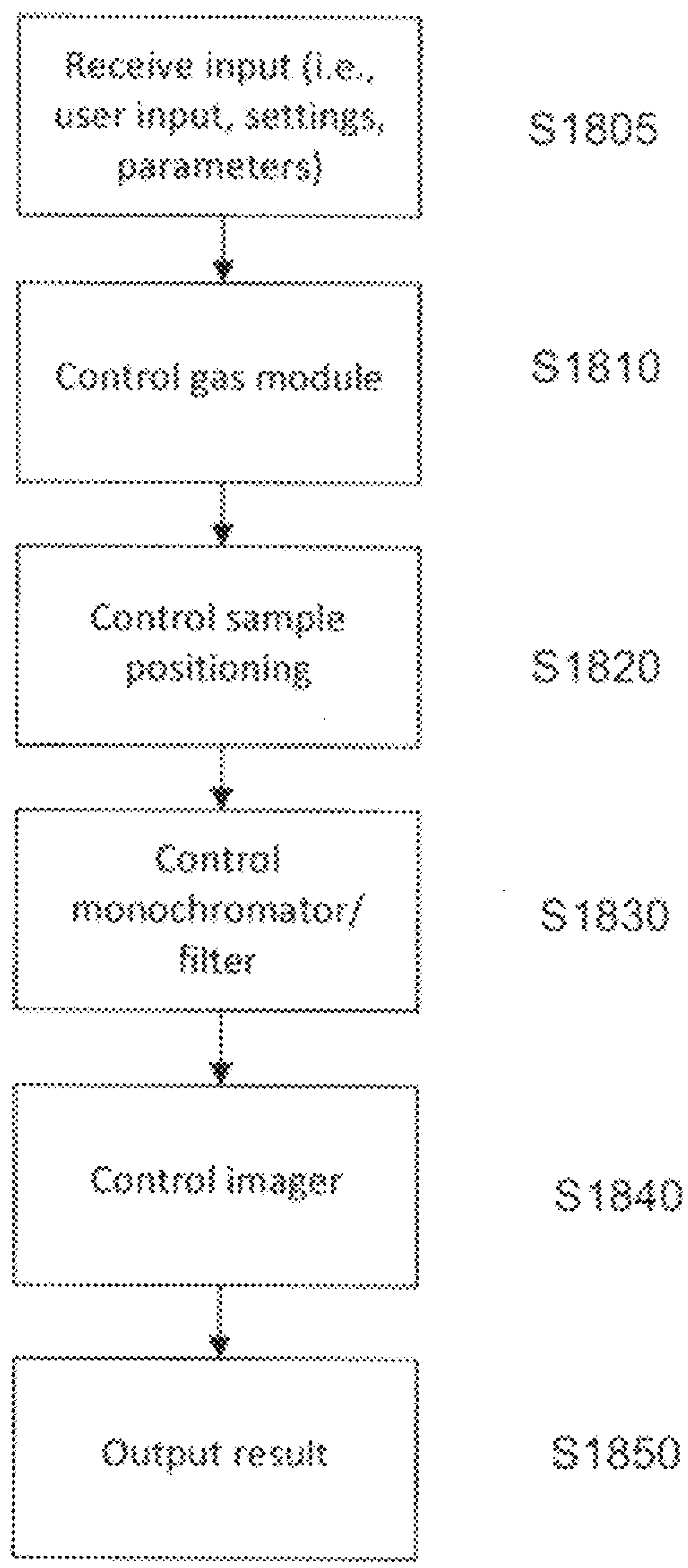

Sample Selection and Positioning
Y motor
X motor
Processor
Sample Environment
Gas control
T control
Imager Module
Focus drive
Imager
LED selector and control
Objective selector
Camera
Filter Module
Filter PMT
Filter selector
Flash lamp
Monochromator Module
Monochromator emission
Monochromator PMT
Monochromator excitation
Flash lamp

FIG. 17

FIG. 18


Receive input (i.e., user input, settings, parameters)
S1805
Control gas module
S1810
Control sample positioning
S1820
Control monochromator/ filter
S1830
Control imager
S1840
Output result
S1850

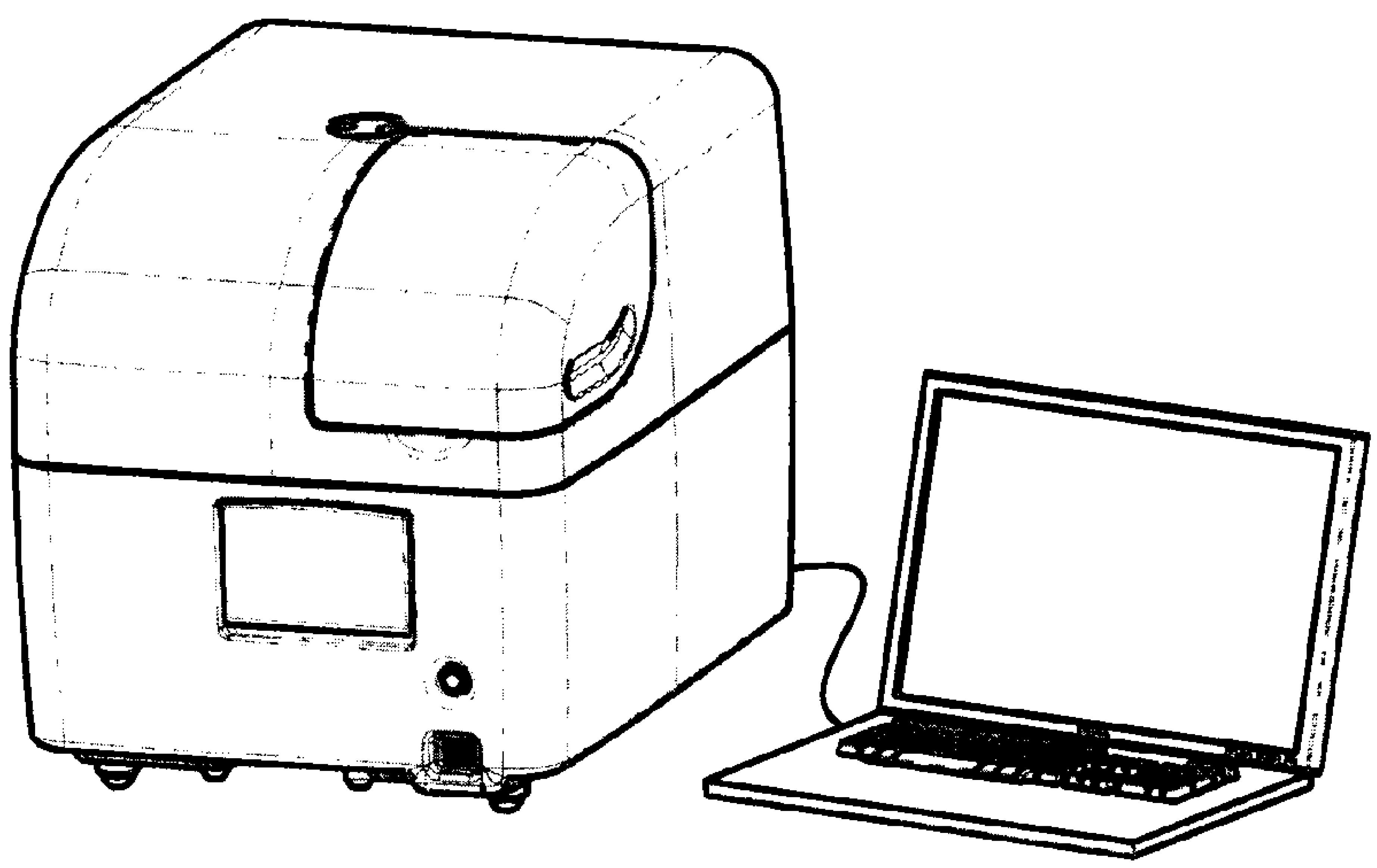


FIG. 19

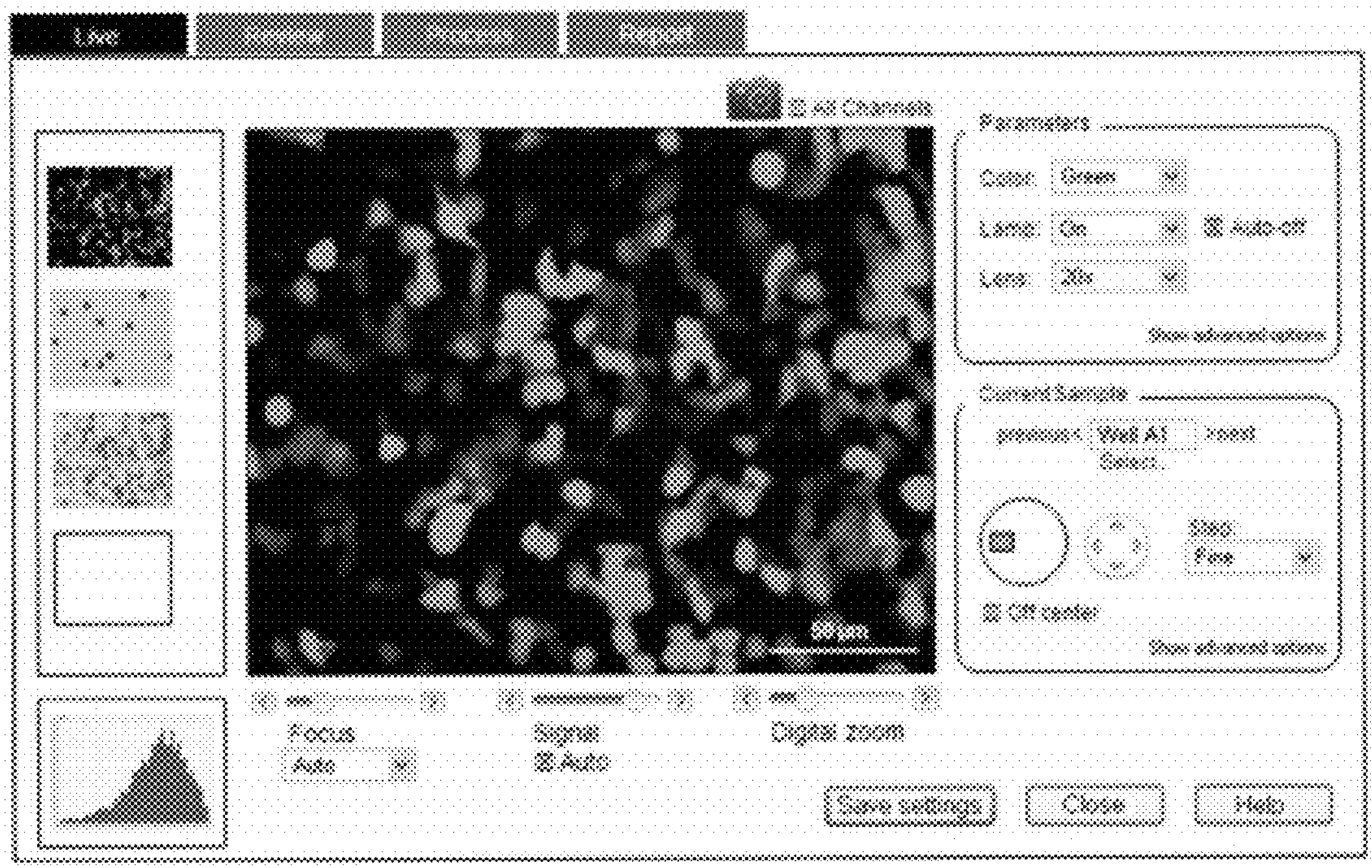

Live
All Channels
Parameters
Color: Green
Lamp: On
Auto-off
Lens: 20x
Show advanced options
Current Sample
Well A1
Select
Step
Fine
Off center
Show advanced options
Focus
Auto
Signal
Auto
Digital zoom
Save settings
Close
Help

FIG. 20

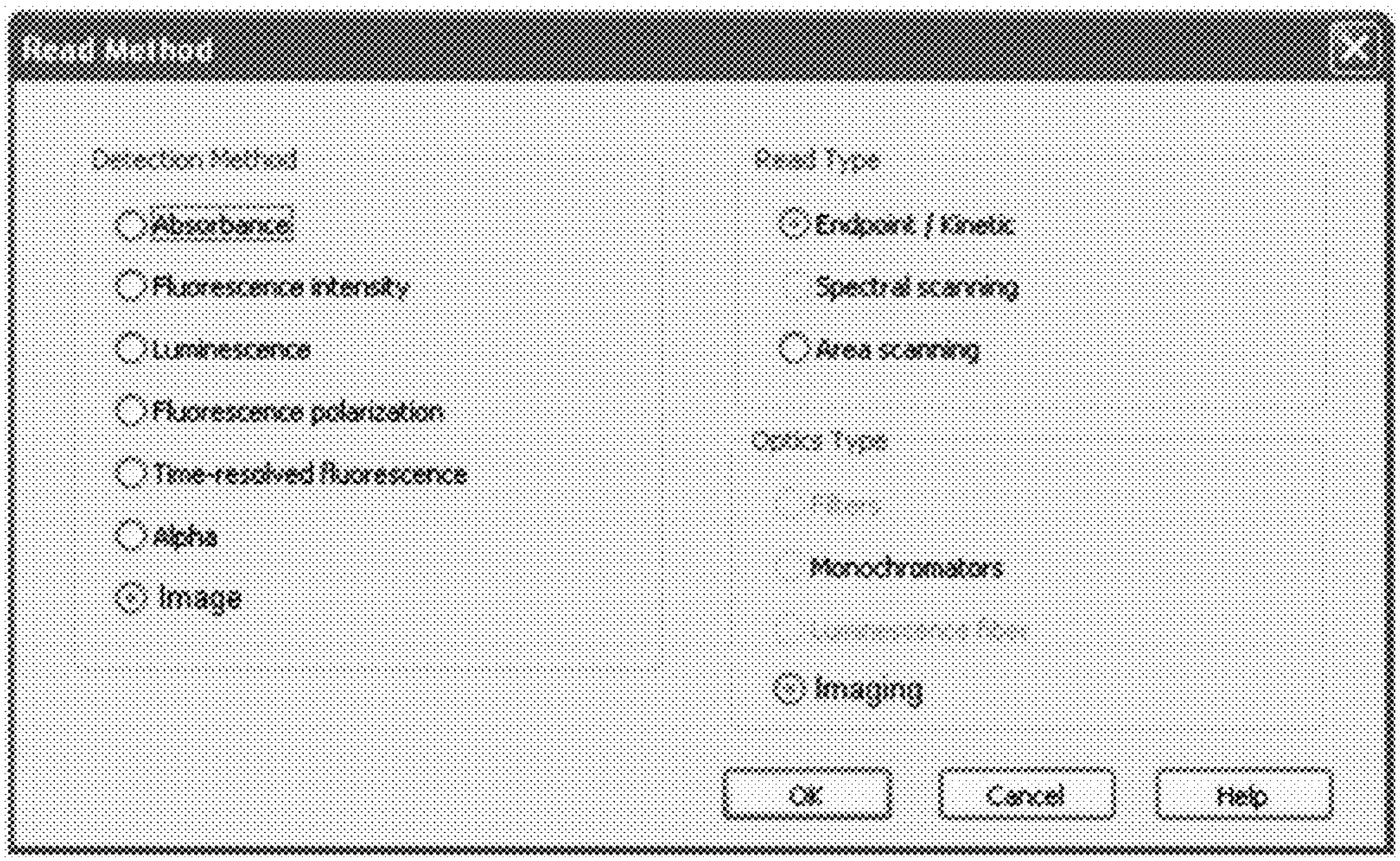

Read Method
Detection Method
Absorbance
Fluorescence intensity
Luminescence
Fluorescence polarization
Time-resolved fluorescence
Alpha
Image
Read Type
Endpoint / Kinetic
Spectral scanning
Area scanning
Optics Type
Monochromators
Imaging
OK
Cancel
Help

FIG. 21

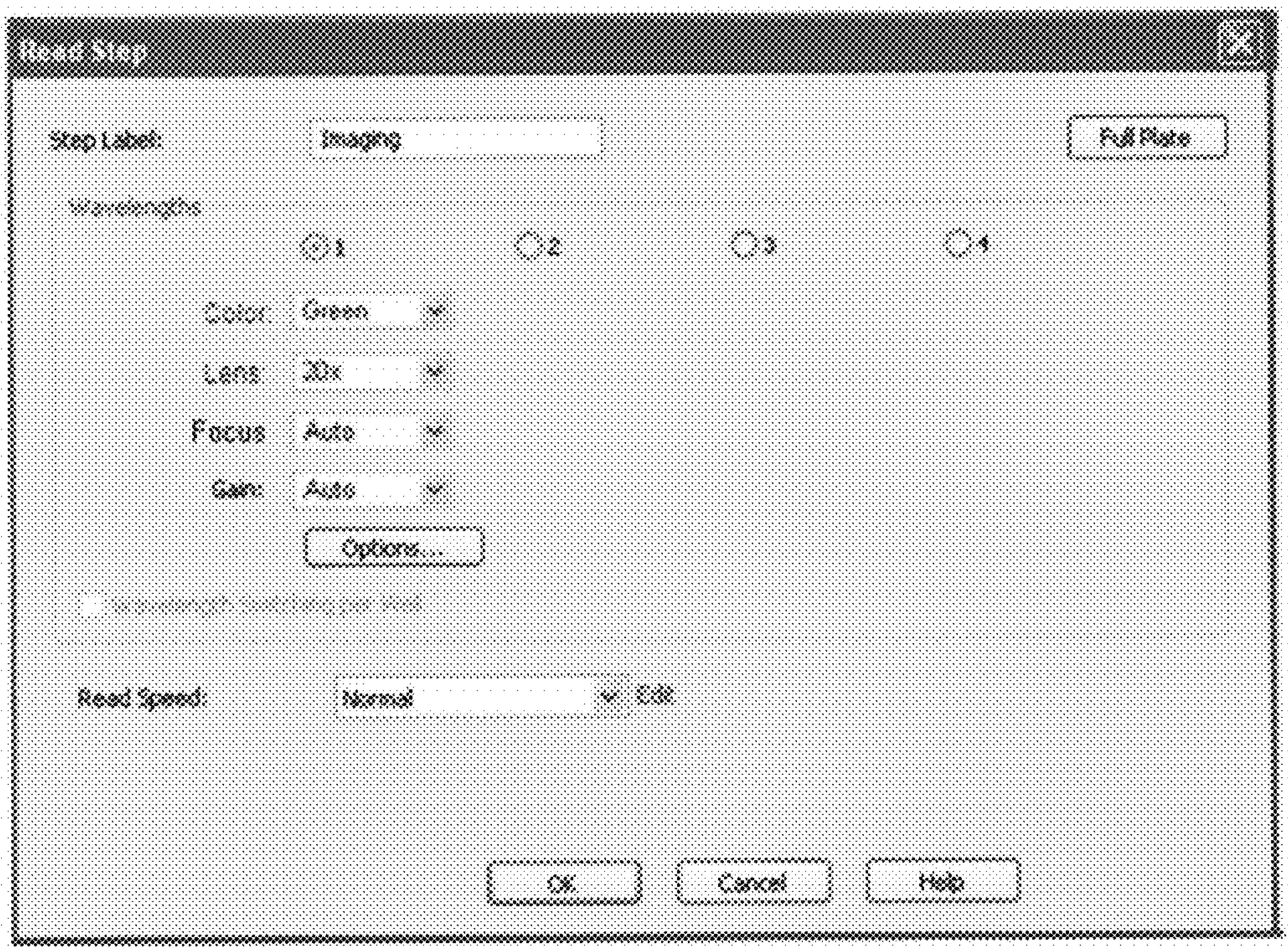

Read Step
Step Label:
Imaging
Full Plate
Wavelengths
1
2
3
4
Color:
Green
Lens:
20x
Focus
Auto
Gain:
Auto
Options...
Read Speed:
Normal
Edit
OK
Cancel
Help

FIG. 22

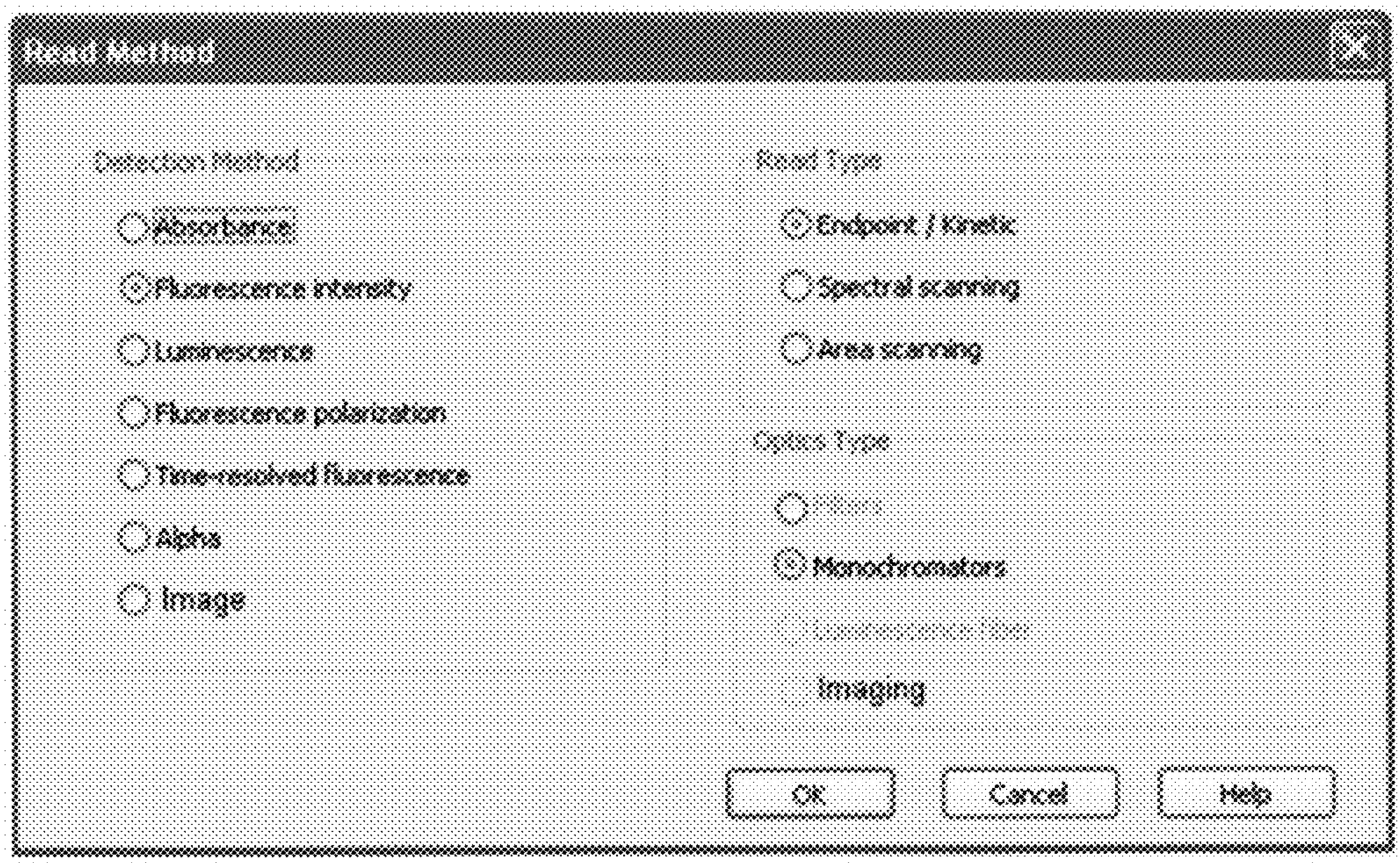

Read Method
Detection Method
Absorbance
Fluorescence intensity
Luminescence
Fluorescence polarization
Time-resolved fluorescence
Alpha
Image
Read Type
Endpoint / Kinetic
Spectral scanning
Area scanning
Optics Type
Monochromators
Imaging
OK
Cancel
Help

FIG. 23

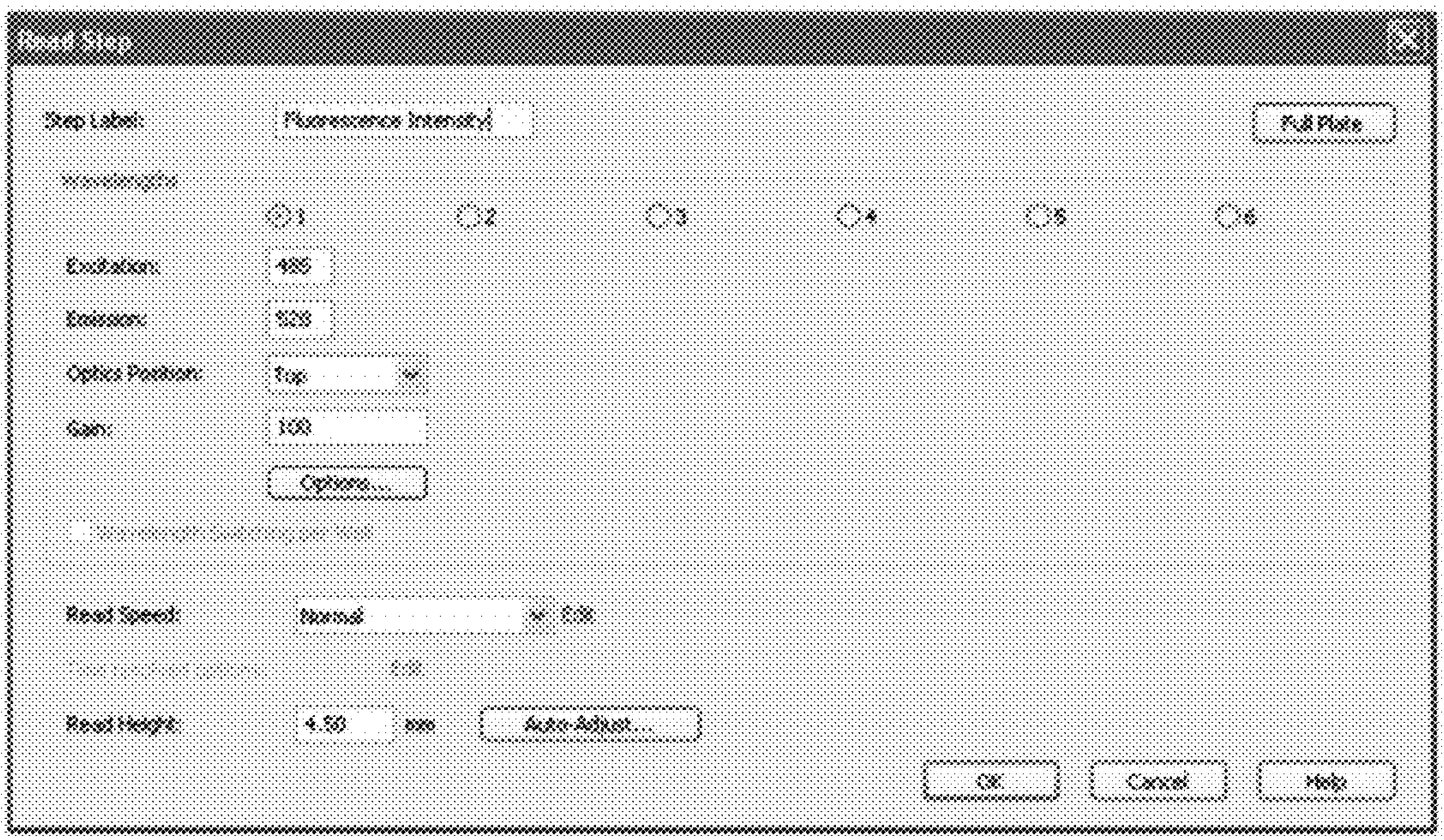

Read Step
Step Label:
Fluorescence Intensity
Full Plate
1
2
3
4
5
6
Excitation:
485
Emission:
528
Optics Position:
Top
Gain:
100
Options...
Read Speed:
Normal
Read Height:
4.50
mm
Auto-Adjust...
OK
Cancel
Help

FIG. 24

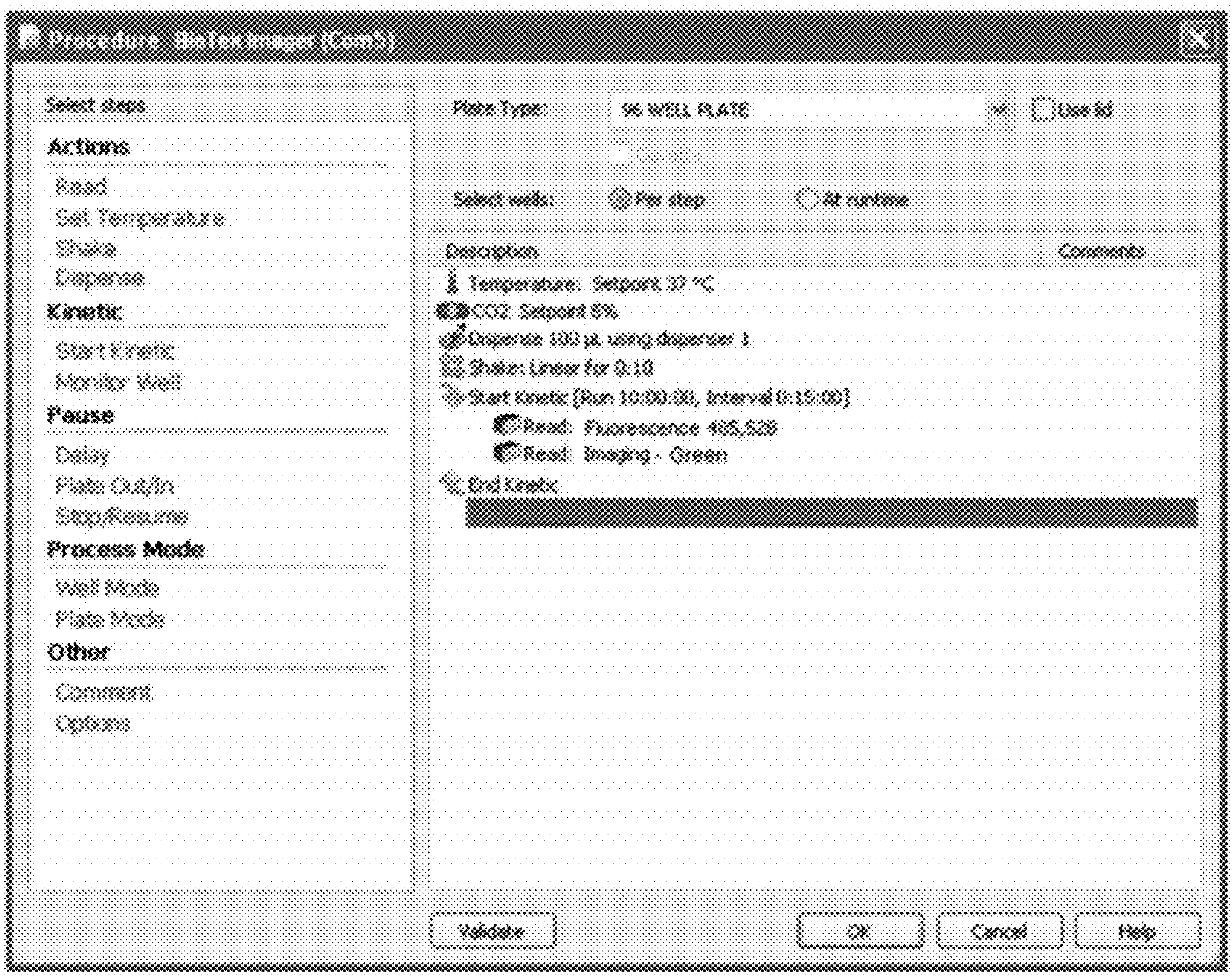

Select steps
Actions
Read
Set Temperature
Shake
Dispense
Kinetic
Start Kinetic
Monitor Well
Pause
Delay
Plate Out/In
Stop/Resume
Process Mode
Well Mode
Plate Mode
Other
Comment
Options
Plate Type:
96 WELL PLATE
Use lid
Select wells:
Per step
At runtime
Description
Comments
Temperature: Setpoint 37 °C
CO2: Setpoint 5%
Shake: Linear for 0:10
Start Kinetic [Run 10:00:00, Interval 0:15:00]
Read: Fluorescence 485,528
Read: Imaging - Green
End Kinetic
Validate
OK
Cancel
Help

FIG. 25

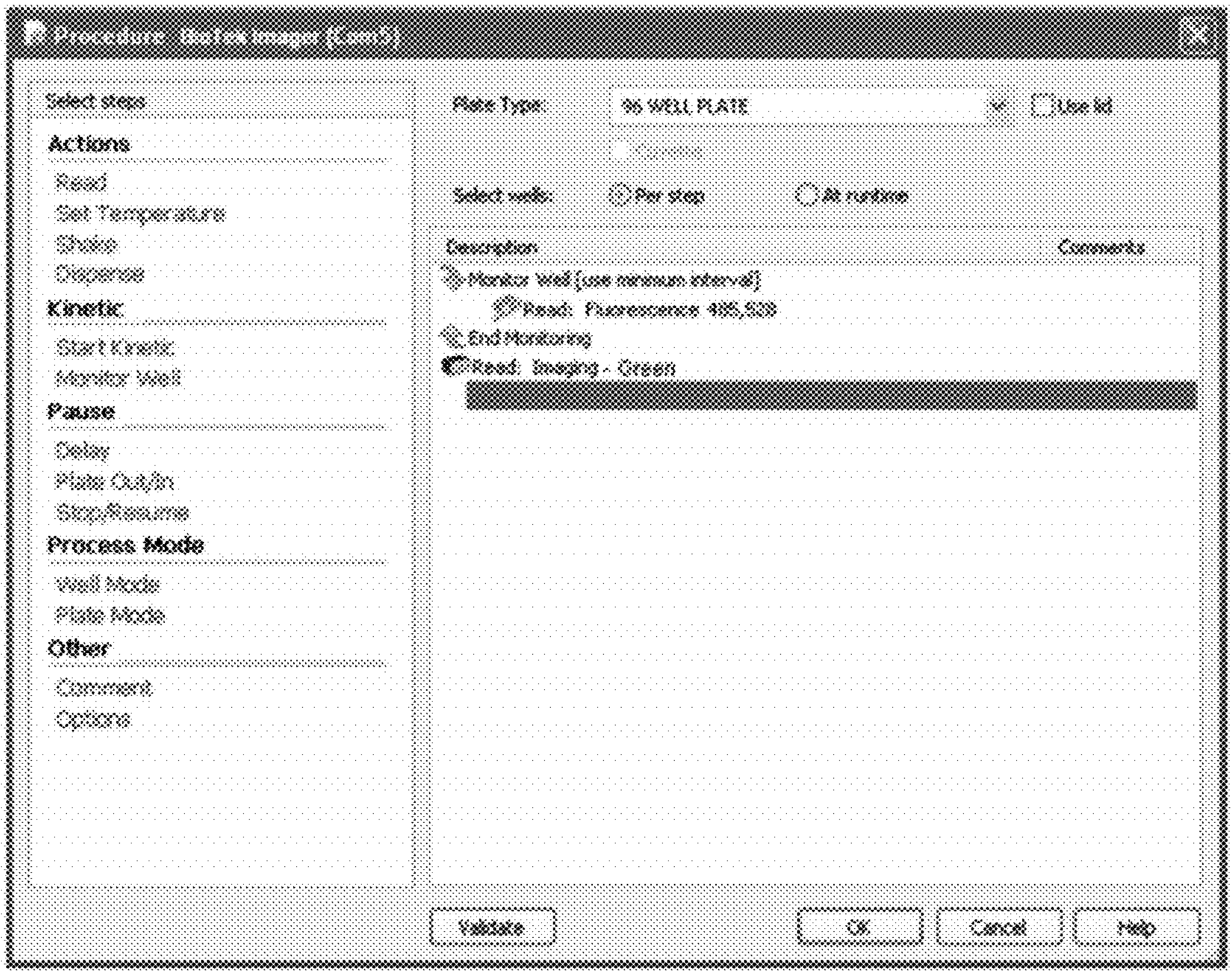

Procedure - Before Image (Cont'd)
Select steps
Actions
Read
Set Temperature
Shake
Dispense
Kinetic
Start Kinetic
Monitor Well
Pause
Delay
Plate Out/In
Stop/Resume
Process Mode
Well Mode
Plate Mode
Other
Comment
Options
Plate Type:
96 WELL PLATE
Use lid
Select wells:
Per step
At runtime
Description
Comments
Monitor Well [use minimum interval]
Read: Fluorescence 485,528
End Monitoring
Read: Imaging - Green
Validate
OK
Cancel
Help

FIG. 26

FIG. 27

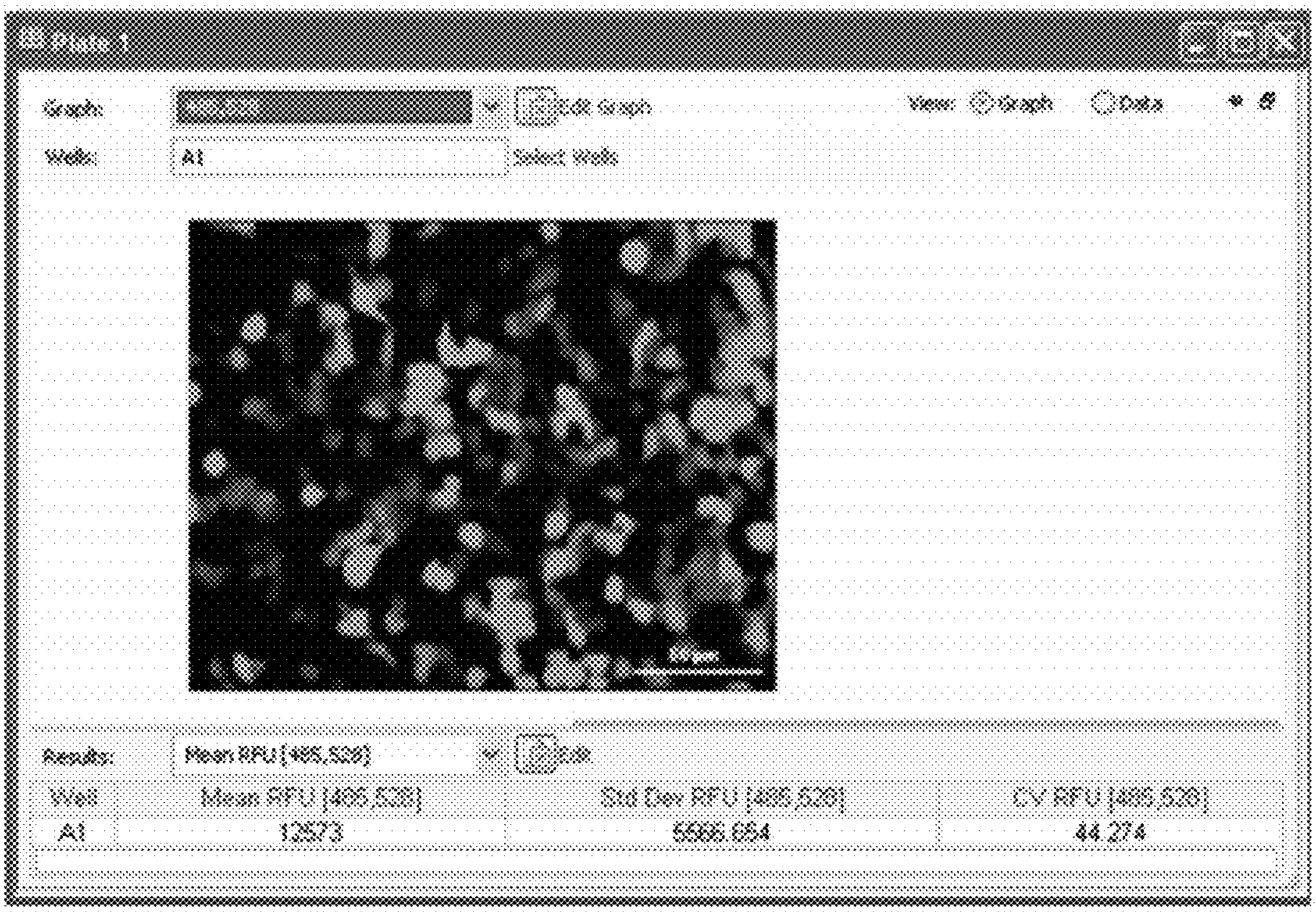


Plate 1
Graph:
Edit Graph
View:
Graph
Data
Wells:
A1
Select Wells
Results:
Edit
Well
Mean RFU
Std Dev RFU
CV RFU
A1
12573
44.274

FIG. 28

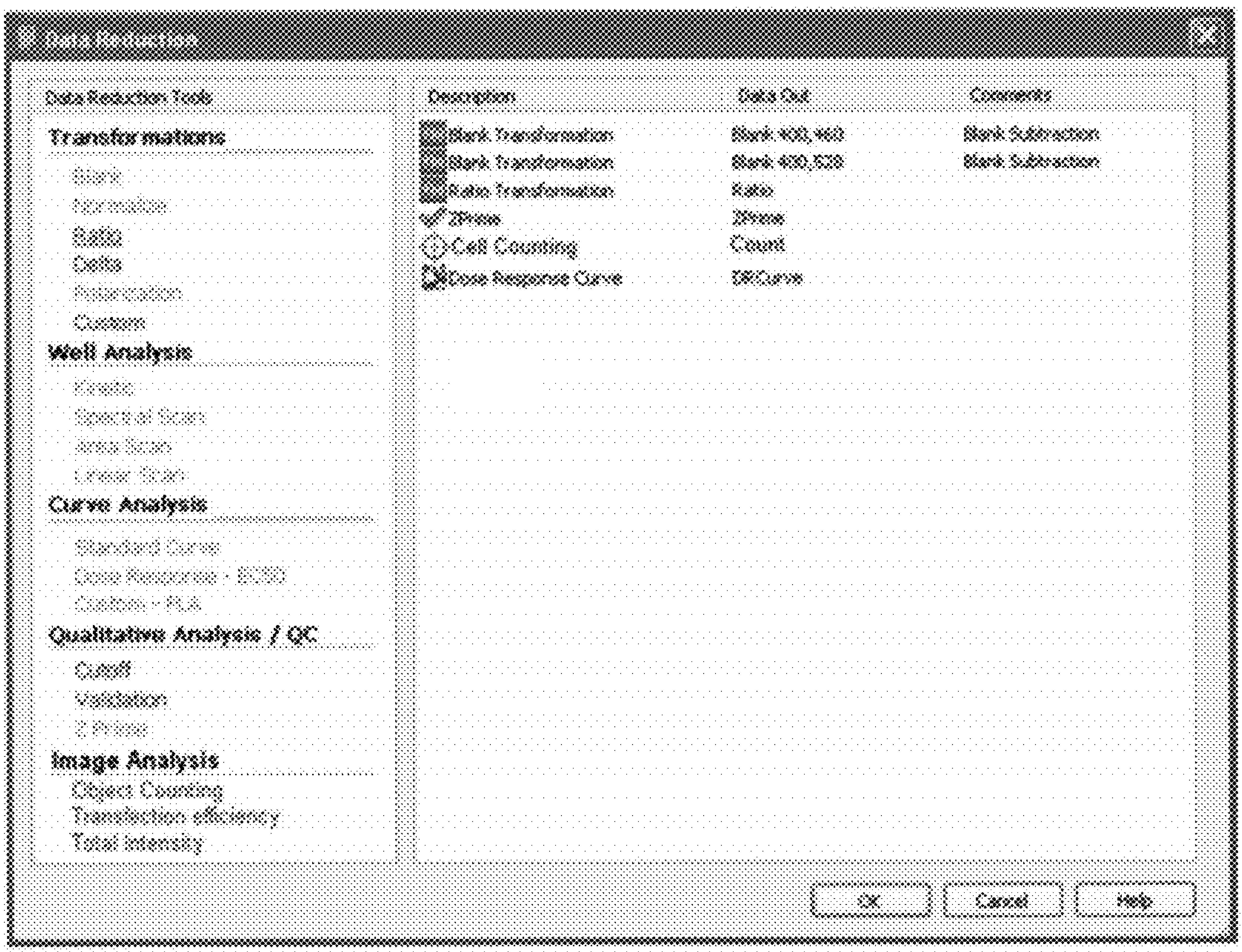

Data Reduction
Data Reduction Tools
Transformations
Blank
Normalize
Ratio
Delta
Polarization
Custom
Well Analysis
Kinetic
Spectral Scan
Area Scan
Linear Scan
Curve Analysis
Standard Curve
Dose Response - EC50
Custom - PLA
Qualitative Analysis / QC
Cutoff
Validation
Z Prime
Image Analysis
Object Counting
Transfection efficiency
Total Intensity
Description
Data Out
Comments
Blank Transformation
Blank 400, 460
Blank Subtraction
Blank Transformation
Blank 400, 520
Blank Subtraction
Ratio Transformation
Ratio
ZPrime
ZPrime
Cell Counting
Count
Dose Response Curve
DRCurve
OK
Cancel
Help

FIG. 29

UNIVERSAL MULTIDETECTION SYSTEM FOR MICROPLATES

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of U.S. application Ser. No. 12/838,804 filed on Jul. 19, 2010, which is a divisional of U.S. application Ser. No. 11/802,831, filed May 25, 2007, which issued as U.S. Pat. No. 7,782,454, the disclosures of which are incorporated by reference in their entireties.

BACKGROUND

1. Field

Apparatuses and methods consistent with the present invention relate to detection systems, including the detection of fluorescence, absorbance, and chemiluminescence in samples placed in the wells of microplates.

2. Description of the Related Work

Multiple analytical instruments are used in laboratories to evaluate samples under test that are placed into vessels of various shapes. In the past twenty years, a microplate format has become very popular, as it lends itself to testing many samples on a single matrix-style receptacle. The first detection systems for microplates were absorbance readers. Later dedicated fluorometers were developed, followed by instruments to measure chemiluminescence.

The range of assay chemistries and labeling technologies continues to grow. Currently employed detection methods include absorbance, multiplexed fluorescence and chemiluminescence, fluorescence polarization (FP), time-resolved fluorescence (TRF), fluorescence resonance energy transfer (FRET), quenching methods, and specially designed labels with intensity and spectral responsiveness to environmental conditions. Along with this range of detection methods, users are conjugating an ever-growing array of organic and inorganic labels for targets, ranging from small-molecule drug candidates to proteins and nucleic acids, and to subcellular structures and cells.

FIG. **1** illustrates the general structure of a related art multimode detection system. As shown in FIG. **1**, a typical system comprises a light source **10**, an excitation spectral device **20**, an optical module **30**, a measurement chamber **60** with samples **70**, an emission spectral device **40**, and a detector **50**. There are two distinct types of related art multimode detection systems: filter-based units and monochromator-based units.

Filter-based units, when offered with high quality filters in combination with dichroic mirrors, allow for measurements with very low detection limits. This is mainly due to a high signal level, which is achieved with the filters, in combination with a high signal-to-noise ratio, which is achieved by a high level of blocking of the unwanted radiation around the desired waveband. The transmittance of filters is routinely over 50%, and this high level of transmittance can be achieved independent of the wavelength. Therefore, a very broad spectral range can be covered equally well from the deep ultraviolet (UV) to the infrared (IR), and the bandpass of the filter can be tailored to the specific application.

However, the filter-based unit cannot obtain a spectral scan for excitation or emission of the substance under investigation. A user must know upfront what substance he or she is working with and order an appropriate filter set. In addition, when working in the deep UV, filters tend to degrade when exposed to the UV radiation of the light source, due to solarization. Also, maintaining libraries of filters for the full range of labels is prohibitively expensive, and appropriate combinations are often not readily available for a given label, conjugation chemistry, target molecule, and assay condition. Further, the effects of these conditions are not always predictable based on the nominal spectra of the basic label.

Monochromator-based instruments offer a high level of flexibility in terms of choosing the wavelengths and obtaining scans of excitation and emission spectra, thus allowing the user to work with unknown substances. This also permits optimization of the measurements for perturbations to the spectra of labels due to assay conditions, conjugation chemistries, and target molecules. Additionally, when working with real biological or biochemical samples, interfering signals from other sample components may require optimization of excitation and emission wavelengths for the exact assay conditions.

The monochromators used in modern instruments are usually based on diffraction gratings, and use a flat grating for dispersion and concave mirrors for focusing light, or concave gratings that combine dispersive and focusing functions. Monochromators require order sorting filters to separate high spectral orders, but in the range from 200 nm to about 380 nm, no order sorting filters are needed. Therefore, there is no need for filters that withstand UV radiation, and the solarization problem is avoided.

However, the response of the monochromator is not constant across the wavelength range. One can obtain a system with a good signal in the UV, the visible, or the IR; however, one cannot obtain a system with a good signal in all of the wavelength ranges in the same monochromator-based unit. A usual compromise is to optimize the excitation monochromator in the UV and to optimize the emission monochromator in the visible or IR, because the wavelength of the emission light shifts to the right with respect to the wavelength of the excitation light.

In order to obtain low detection limits, the monochromator must have very low stray light. A traditional way to achieve this in the monochromator-based system is to employ two stage monochromators. These are called double monochromators, and contain two single monochromators placed in series. While this does result in very low stray light, the penalty is a dramatic decrease in signal, especially in spectral regions where the response of the single stage monochromator is already low. There are several instruments in the field based on this method.

In terms of performance, the filter-based units achieve significantly lower detection limits in fluorescence intensity applications across the full spectral range, and work significantly better with techniques such as TRF, FP, and Homogeneous Time-Resolved Fluorescence (HTRF), all of which require the strong signal provided by the filter-based units. On the other hand, the monochromator-based units provide the flexibility of choosing any wavelength and the ability to obtain a spectral scan, at the expense of lower sensitivity.

U.S. Pat. No. 6,313,471 describes a method that combines bandpass filters and monochromators in series in a detection system. In this method, the bandpass filter acts as a crude first stage monochromator. The instrument splits the full spectral range of interest into several regions corresponding to the number of filters employed, and blocks radiation from adjacent regions by using additional filters. The single stage monochromator that follows the bandpass filters then selects the wavelength of interest from this prefiltered range.

However, with a limited number of prefiltered regions, this method is limited in flexibility. If both the excitation and emission wavelengths fall into one region, the method is not effective in achieving low stray light or high performance. True spectral scanning is not readily accomplished with this method. This limits its utility for spectral measurement and optimization under conditions of fine spectral perturbation.

A most recent advance in microplate instrumentation is a multi-detection analyzer. An example of this product is the Synergy line from BioTek Instruments. The included modalities are absorbance, fluorescence, luminescence, and fluid injection.

There is a desire to study cellular processes in microplates, and thus the need to visually study the contents of the microwells. Accordingly, a synergistic effect would be obtained by combining in one instrument the ability to perform imaging of the wells of the microplates and reading modality, such as absorbance, luminescence, or high sensitivity fluorescence readings.

SUMMARY

Exemplary embodiments described herein overcome the above disadvantages and other disadvantages not described above. Also, the exemplary embodiments are not required to overcome the disadvantages described above, and an exemplary embodiment may not overcome any of the problems described above.

According to an aspect of an exemplary embodiment, there is provided a device for analyzing one or more samples, the device including a support for a receptacle that holds a sample; an imaging subsystem that images the sample; and an analyzing subsystem that analyzes the sample.

According to an aspect of an exemplary embodiment, there is provided a sample analysis method including selecting at least one subsystem from among a plurality of subsystems of a sample analysis device that examines one or more samples, the plurality of subsystems comprising an imaging subsystem that images the one or more samples and an analyzing subsystem that analyzes the one or more samples; and controlling the selected at least one subsystem to perform an examination on the one or more samples, the examination comprising an imaging operation of the imaging subsystem that images the one or more samples and an analyzing operation of the analyzing subsystem that analyzes the one or more samples.

According to an aspect of an exemplary embodiment, there is provided a non-transitory computer-readable medium having embodied thereon a program which when executed by a computer causes the computer to execute a sample examination method, the method including selecting at least one subsystem from among a plurality of subsystems of a sample analysis device that examines one or more samples, the plurality of subsystems comprising an imaging subsystem that images the one or more samples and an analyzing subsystem that analyzes the one or more samples; and controlling the selected at least one subsystem to perform an examination on the one or more samples, the examination comprising an imaging operation of the imaging subsystem that images the one or more samples and an analyzing operation of the analyzing subsystem that analyzes the one or more samples.

BRIEF DESCRIPTION OF THE DRAWINGS

The above and other aspects will become more apparent by describing in detail exemplary embodiments thereof with reference to the attached drawings, in which:

FIG. **1** illustrates a general structure of a multimode detection system;

FIG. **2** illustrates certain components of a Universal Multi-detection System (UMS) according to an exemplary embodiment of the present invention;

FIG. **3** illustrates a light source according to an exemplary embodiment of the present invention;

FIG. **4** illustrates an excitation spectral device according to an exemplary embodiment of the present invention;

FIG. **5** shows optical connections between the light source, the excitation spectral device, and the excitation-emission separation device according to an exemplary embodiment of the present invention;

FIG. **6** illustrates an excitation-emission separation device according to an exemplary embodiment of the present invention;

FIG. **7** illustrates a holder according to an exemplary embodiment of the present invention;

FIG. **8** shows a view of the excitation-emission separation device along vertical axes toward the microplate according to an exemplary embodiment of the present invention;

FIG. **9** illustrates an emission spectral device according to an exemplary embodiment of the present invention; and

FIG. **10** shows a fluid dispenser according to an exemplary embodiment of the present invention.

FIG. **11**A illustrates a UMS according to an exemplary embodiment, in which an imaging subsystem is included in the UMS.

FIG. **11**B illustrates a UMS according to an exemplary embodiment, in which an imaging subsystem is included in the UMS.

FIGS. **12** and **13** illustrate a UMS according to an exemplary embodiment, in which atmospheric control is implemented.

FIG. **14** illustrates an LED module of an imager, according to an exemplary embodiment.

FIG. **15** illustrates a turret having objectives of the imaging module, according to an exemplary embodiment.

FIG. **16** illustrates views of a gas control module, according to an exemplary embodiment.

FIG. **17** is a functional block diagram that illustrates control of modalities, according to an exemplary embodiment.

FIG. **18** is a flowchart of a control method of a UMS, according to an exemplary embodiment.

FIG. **19** illustrates a control apparatus for controlling a UMS, according to an exemplary embodiment.

FIG. **20** illustrates operations of an imaging subsystem of a UMS, according to an exemplary embodiment.

FIG. **21** illustrates mode selection of operations of a UMS, according to an exemplary embodiment.

FIG. **22** illustrates parameter settings of an imaging subsystem of a UMS, according to an exemplary embodiment.

FIG. **23** illustrates parameter settings of an analysis subsystem of a UMS, according to an exemplary embodiment.

FIG. **24** illustrates parameter settings of an analysis subsystem of a UMS, according to an exemplary embodiment.

FIG. **25** illustrates settings of multiple subsystems of a UMS, according to an exemplary embodiment.

FIG. **26** illustrates monitoring operations by an analysis subsystem and conditional imaging by an imaging subsystem of a UMS, according to an exemplary embodiment.

FIG. **27** illustrates results of operations of an analysis subsystem and an imaging subsystem of a UMS, according to an exemplary embodiment.

FIG. **28** illustrates a detailed result of operations of an analysis subsystem of a UMS, according to an exemplary embodiment.

FIG. **29** illustrates data reduction options for combined analysis and imaging subsystems of a UMS, according to an exemplary embodiment.

DETAILED DESCRIPTION OF EXEMPLARY EMBODIMENTS

Cell-based and live-cell assays are becoming more and more popular in life science research and drug discovery as the field of biology keeps developing in depth and complexity. Cells are complex biological entities and multi-parametric, multiplexed assays are becoming more common (for example, assay measuring 3 cellular events in parallel). The ability to monitor live cells using multi-parametric assays is key to developing a better understanding of cell biology.

Assays may be conducted with microplate multimode readers, using filters or monochromators, which collect as much signal as possible from the microplate well using the full population of cells. These assays are typically quantitative and the signal is an average produced by the full population of cells. Other assays use microplate imaging readers that contain microscope objectives and a camera to image a small portion of the well and thus a sub-population of cells. This provides the ability to localize where the signal is originating in individual cells and provide semi-quantitative information from the extent of the signal compared to controls. Another way of differentiating these assays is whether they involve end-point or kinetic responses.

One benefit of a hybrid instrument is to dramatically increase the ability to get multi-parametric data from live-cell assays. In particular, this novel instrument design allows making population-based detection synchronous with single-cell results available from imaging. 2D imaging information produces feedback about individual cell behaviors within the population. This allows the simultaneous study of populations and single cells, which will identify sub-populations and better characterize the biology and drug effects.

This important benefit is especially evident in the case of kinetic assays. Cellular events occur over a certain period of time (minutes, hours or days depending on type of event) and kinetic monitoring allows recording changes over time. Only a hybrid system as described herein will allow monitoring in parallel population-based information with the microplate reader optics and single cell-based information with the imaging optics, providing a very detailed view of what is happening in the sample. Such an assay may be one in which cell viability and cell death are measured. This assay is typically measured with standard plate readers and the instrument acquires a signal coming from the solution in which the cells grow. Such a signal cannot be satisfactorily measured using imaging optics. On the other hand, these indirect signals are assumed to correspond to cell death and cell viability events but only direct visual inspection can confirm what is happening at the cellular level. Monitoring both chemical signals in solution with the plate reader optics and actual cell morphology changes with the imaging optics enhances the quality of the data set and provide more information on the actual cellular events.

Another unique benefit of a hybrid microplate reader defined as both a microplate multimode reader and microplate imaging reader is significant instrumentation cost savings and workflow improvement. This is due to the ability to automatically perform both end-point and kinetic assays on both sub- and full populations of cells in the same sample, during the same experiment. Normally, this would require at least two separate instruments. An example of this would be where a GFP fusion protein with Histone H3 is created using the Bacmam transfection technology. Histones are located exclusively in the cell nucleus, so imaging of sub-populations of cells using the imaging part of the hybrid reader is a useful validation step to ensure that green fluorescence from GFP is located exclusively in the nucleus. The determination of the extent of histone deacetylation at the lysine 9 residue of histone H3 is then conducted as an end-point assay using a labeled antibody against histone H3 (lysine 9) on the full population of cells in the well using the optics of a microplate multimode reader in the hybrid instrument.

Yet another unique benefit is the ability to improve on two major drawbacks of imaging: read speed and amount of data generated. Imaging takes longer than acquiring one data point per sample, and each individual sample file can be 1 MB or more. An instrument in accordance with aspects of the present application will allow quickly scanning dozens or hundreds of samples, identifying the samples of interest then limiting imaging to these identified samples. This process will significantly reduce the total acquisition time and as well as the final size of the data set.

The combination of an imaging subsystem and an analyzing subsystem permits benefits in many areas, including those already discussed and those discussed below.

a. Cell Counting

The purpose of cell counting in microplate assays is to estimate the increase or decrease of a cell population after treatment with a molecule of interest. Cytotoxicity assays are intended to measure population decline while cell proliferation assays are designed to detect population growth. These assays are among the most common cell-based microplate assays run in life-science laboratories. In conventional microplate readers, cell counting is performed using assays that generate a signal proportional to the number of cells. One of the most common such assay is a luminescent ATP assay: at the time of measurement, the cells are lysed (the cell membrane is destroyed so that the content of the cell is released), and ATP (energy-storage molecule found in all living cells) concentration is measured using a reagent that generates light in the presence of ATP. ATP concentration is proportional to the number of cells, and as a result the luminescent signal is proportional to the number of cells prior to analysis. Since this is a destructive assay, it can only be run once on each sample. There are instances where this type of assay can produce unexpected results.

The imaging subsystem permits scientists to take a quick look under a microscope before processing the microplate, to ensure that that cell population looks as expected. Accordingly, a second data point may be obtained for data analysis. Thus, for each sample, the user would have two sets of data: an image giving qualitative and semi-quantitative (estimate) information about the cell population, and a quantitative signal once the ATP assay has been run. The two sets of data would be expected to match in most cases (confirmation test), but a disagreement between the image and the quantitative assay would be a critical piece of information.

b. Transfection Efficiency and Gene Expression Assays

The combination of an imaging subsystem and an analyzing subsystem enables documenting the history of the cell population using one software and one instrument during transfection, which is a practice in modern laboratories to add genetic material to cells for the purpose of studying specific genes.

For example, once the transfection has been accomplished and cells have had time to recover from the process, the efficiency of the transfection step is estimated using the image subsystem to determine how many cells among the total cell population have effectively incorporated the new gene. Once this has been established, and if the transfection efficiency is high enough, researchers can then carry on with their experiments and will often run assays on conventional microplate readers.

c. Sample Documentation

The combination of an imaging subsystem and an analyzing subsystem enables sample information (qualitative and quantitative) to be regrouped in one electronic file, which will give researchers better and easier access to their sample data, allow researchers to easily see multi-dimensional data related to one sample, and thereby help bring clarity to the data-heavy cell-based research environment.

For example, large numbers of assay may rely on fluorescently labeled biological samples (cells, tissues, microscopic worms and fish, . . .) to study the mechanisms of life. The imaging subsystem enables measuring data points on an entire population or organism by measuring the total fluorescence coming from a specific sample, which provides a quantitative answer. Further, qualitative data may be obtained to determine where fluorescence is located, whether the samples look as expected (number of cells, shape, distribution . . .), etc., which permits better documentation of the sample.

The foregoing and other benefits attributable to the present application are particularly important for "Systems Biology", which is a concept at the core of modern biomedical research and has grown significantly since the year 2000. Systems Biology focuses on complex interactions in live biological systems, and relies heavily on a proper experimental model. The cell represents one of these models that is increasingly used to study and understand more complex Systems Biology questions that cannot be answered by simple mix-and-read assays. For this reason, proper instrumentation and software tools are essential for biologists running cell-based and live-cell assays.

When working on live cells, it is important to control gas levels, in particular CO_2 concentration for the purpose of maintaining close-to-physiological conditions. Cells being living organisms, they are sensitive to, and can react to, changes in their environment, such as changes in gas concentrations or changes in temperature conditions. Changes in the gas conditions and thus pH of growth media have even been linked to change in gene expression.

The ability of systems in accordance with aspects of the present application to maintain stable, continuous conditions while switching detection technology from imaging, to filter-based, to monochromator-based, allows monitoring multiple qualitative and quantitative cellular events while maintaining stable cell culture conditions. A benefit for users is the ability to precisely control and maintain optimal conditions on one instrument platform, instead of having to manually move samples from device to device, which could induce unexpected experimental artifacts (e.g. cells reacting to temperature change, mechanical movements or gas concentration changes while being transported from one device to the other).

Thus gas control in accordance with aspects of the present application will provide more reliable, physiologically relevant results in both short term and long term studies in which the same plate might be read many times over a time course. In this case the plate could remain in the instrument and not be subjected to multiple cycles of climate and gas changes.

FIG. **2** illustrates certain components of a Universal Multi-detection System (UMS) **100** according to an exemplary embodiment of the present invention. As shown in FIG. **2**, samples are dispensed into the array of microwells **200** in the microplate **300**. The microplate **300** is transported by the carriage **310** into the measurement chamber **320**, which may be incubated, and is positioned sequentially for measurements. The light source **400** generates excitation light. The excitation spectral device **500** selects and transmits a narrow band of the excitation light. The waveband is typically between 5 and 40 nm wide. The excitation-emission separation device **600** directs the excitation light to the microwells **200**, and then separates the emission light generated in the sample within the microwells **200** from the excitation light. The excitation-emission separation device **600** transmits the emission light to the emission spectral device **700**, which transmits a narrow band of the emission light. The emission spectral device **700** should be configured to transmit as much emission light as possible, while blocking as much excitation light as possible and maximizing the signal-to-noise ratio. The detector **800** converts the emission light into an electrical signal. Although the light source **400**, the excitation spectral device **500**, the excitation-emission separation device **600**, the emission spectral device **700**, and the detector **800** are shown as separate modules, they can also be combined in a variety of ways.

As shown in FIG. **2**, relay devices **910**, **920**, **930**, and **940** provide optical connections between the light source **400**, the excitation spectral device **500**, the excitation-emission separation device **600**, the emission spectral device **700**, and the detector **800**. The controller **1000** stores emission signals from samples in the microplate **300**, analyzes the emission signals, computes parameters categorizing the optical measurements, and sends commands to the light source **400**, the excitation spectral device **500**, the excitation-emission separation device **600**, the emission spectral device **700**, or the detector **800**. The commands can instruct the light source **400**, the excitation spectral device **500**, the excitation-emission separation device **600**, the emission spectral device **700**, or the detector **800** to change an internal parameter. For example, the commands can instruct the excitation spectral device **500** or the emission spectral device **700** to use one internal device instead of another internal device. Further, an optional dispenser **1100** delivers reagent to the microwells **200**.

FIG. **3** illustrates the structure of the light source **400** according to an exemplary embodiment of the present invention. In a preferred embodiment, the light source **400** comprises only two light generating devices: a Xenon flash lamp **410** and a Tungsten lamp **420**. In other embodiments the light source **400** may comprise a Xenon continuous wave lamp, a light emitting diode (LED), a laser, or any other light-generating device.

Tungsten sources are very stable, and their radiation extends from blue in the visible spectrum to the far IR, and peaks around 1 μm. They are most suitable for measurements in the visible and IR regions of the spectrum. In contrast, Xenon flash sources deliver most of their radiation in the deep UV, UV, and short visible spectral ranges. In addition, Xenon flash sources provide a very fast burst of light, lasting for several microseconds with a fast decay, and are therefore suitable for time resolved measurements in modern multi-detection systems.

The Xenon flash lamp **410** has a parabolic reflector **411** positioned such that the arc **412** of the lamp **410** is located near the focal point of the reflector **411**, providing an essentially collimated beam from the reflector **411**. The Tungsten lamp **420** has a parabolic reflector **421** positioned such that the filament **422** of the lamp **420** is located near the focal point of the reflector **421**, providing an essentially collimated beam from the reflector **421**. FIG. **3** shows that a lens **423** may be used to focus the beam from the reflector **421** onto the exit portal **430** of the light source **400**. As shown in FIG. **5**, relay optics may be used to focus the beam onto the entrance of an optical fiber. Alternatively, the lens **423** may focus the beam from the reflector **421** directly onto the entrance of an optical fiber within the excitation spectral device **500**.

The movable off-axis parabolic reflector **440** has two working locations. In the first location, depicted by a solid line in FIG. **3**, the reflector **440** reflects and focuses light from the reflector **411**. In the second location, depicted by a dashed line in FIG. **3**, the reflector **440** stays out of the way of light from the reflector **421**. This arrangement allows light from either lamp to be focused at the same location. Further, the fan **417** directs air across the fins **415** of a cooling extrusion for the Xenon source **410** and onto the Tungsten source **420**. This arrangement allows both sources to share a single cooling system.

The arrangement of two light sources in close proximity to each other, with their optical axes offset, and preferably at an angle of approximately 90 degrees to each other, allows for a very compact illumination system with a shared cooling system. The use of parabolic reflectors around the light sources, in combination with off-axis parabolic reflectors, results in very highly efficient coupling of light from the arc and filament into the system. Here the final focusing point of both light sources is the same. This system allows a more compact arrangement than a system which utilizes separate light source compartments with separate exit light points for each compartment, thus requiring a mechanical movement of the optical relay system to switch between sources.

FIG. **4** illustrates the structure of the excitation spectral device **500** according to an exemplary embodiment of the present invention. The exit portal **430** of the light source **400** is in close proximity to the input portal **510** of the excitation spectral device **500**. The relay device **910** is unfilled space inside the UMS **100**.

In a preferred embodiment, the excitation spectral device **500** has two spectral selection devices, which differ by the physical technology by which they separate light with different wavelengths. The first device is a filter selection device **520**, which has a variety of user-replaceable filters **521**. The second device is a double monochromator **530**.

As shown in FIG. **4**, the light exiting the light source **400** via the exit port **430** is directed to the entry point **510** of the excitation spectral device **500**. Light entering the excitation spectral device **500** is then directed to the exit port **540** of the excitation spectral device **500** along one of two paths.

The first path directs the light through one of the filters **521** in the filter selection device **520**, which transmits a narrow band of the light. The light then propagates through optical fiber **522** to the exit port **540**. The second path bypasses the filters **521** by directing the light through hole **523** in the filter selection device **520**. The light then continues via optical fiber **531**, which accepts a circular image of the arc or filament spot from the light source **400** formed at the entry port **510**, and shapes the light spot into a slit shape to match it to the input slit of the double monochromator **530**. The monochromator **530** selects a narrow band of the light, and then the optical fiber **532** changes the shape of the light from the exit slit shape of the monochromator **530** into a circular shape that resembles the shape of a microwell **200**.

The light path selector **550** can move relative to the filter selection device **520**, providing the ability to guide light to the exit port **540** that was spectrally selected by the filters **521** or the monochromator **530**. FIG. **5** shows the optical connections between the light source **400**, the excitation spectral device **500**, and the excitation-emission separation device **600** in greater detail.

FIG. **6** illustrates the structure of the excitation-emission separation device **600** according to an exemplary embodiment of the present invention. The general purpose of the excitation-emission separation device **600** is to irradiate the sample with excitation light and/or gather emission light from the sample. The excitation-emission separation device **600** can be positioned above the microwell **200** as shown in FIG. **6**, or below the microwell **200**. Also, the Universal Multi-detection System **100** can include two excitation-emission separation devices **600**, one of which is positioned above the microwell **200**, and the other of which is positioned below the microwell **200**. This arrangement enables measurements of the same microwell **200** with a filter-based system and a monochromator-based system from both the top and the bottom.

In a preferred embodiment, several light paths may be used, based on the measurement technique. For absorbance measurements, the excitation and emission light are preferably collinear. As shown in FIG. **6**, the absorbance measurements are conducted in block **640**, in which the microwell **200** is illuminated with excitation light from below at point G. This excitation light may come from the monochromator **530** or the filter selection device **520**, as shown in FIG. **5**. A detector **650** is placed on the opposite side of the microwell **200** to capture emission light that passes through the sample.

For luminescence measurements, no excitation light is required, and only emission light is gathered from the sample by the excitation-emission separation device **600**. In block **630**, a single fiber optic bundle **735** is used to maximize the light gathering capability of the system and thus improve the signal.

For fluorescence measurements, two optical paths are available to irradiate the sample with excitation light and to gather emission light from the sample. These paths can be optimized to further enhance the overall system performance.

Block **620** depicts a first optical path for fluorescence measurements, which can use a partially reflective mirror or a dichroic mirror so that excitation light and emission light are collinear when entering and exiting the sample, respectively. Light is delivered to Block **620** by the optical fiber **522**. The movable aperture **601** has several openings with diameters preferably ranging from approximately 1.5 mm to 4 mm, and is placed in front of the guide fiber **522**. An image of the opening placed in front of the optical fiber **522** is formed in the microwell **200** by lenses **621** and **622**. The size of the opening of the movable aperture **601** is selected to fill the microwell **200** as completely as possible with light, while preventing light from entering adjacent microwells and causing cross-talk.

The light is reflected by a partially transmitting mirror **623** on a movable holder **627**. More than one mirror can be placed onto the holder **627**. Some mirrors can be dichroic mirrors to improve the signal, as all excitation light is reflected towards the microwell **200**, and all emission light is transmitted towards exit fiber. The dichroic mirrors can also improve the signal-to-noise ratio of the measurement system, as residual excitation light that reaches the microwell **200** and is reflected by the meniscus lens is blocked from reaching the exit fiber. The emission light from the microwell **200** is gathered onto the fiber optic bundle **731** by lenses **621**, **622**, and **670**. A collective lens **670** in front of the fiber optic bundle **731** assures that emission light from the full depth of the microwell **200** is collected, thus maximizing the system signal.

The high energy collection characteristics of the system assure low detection limits and allow for various levels of fluid to produce acceptable results without the need to refocus the optical system based on the fluid volume. This is in contrast with, for example, the confocal style measurements described in U.S. Pat. No. 6,097,025, which uses a confocal optical system that collects light only from the small portion of the microwell.

In a preferred embodiment, linear polarizers **624** and **625** are added to the holder **627**, and the same motion that positions appropriate mirrors in the light path also can be used to select polarizers for fluorescence polarization measurements. This eliminates the need for a separate mechanism to switch the polarizers, and thus improves the reliability of the system.

Block **610** depicts a second optical path for fluorescence measurements, which uses a tilted V arrangement of optics for direct well illumination and light gathering. This allows the system to channel the full amount of light from the fiber optic **532** into the microwell **200**. The numerical aperture of the optics **611** and **612** is matched to the fiber optic **532** for this purpose. The cone of excitation light enters the microwell **200** and excites the contents of the microwell **200** via the first leg of the V. The emission light is collected by the second leg of the V. The numerical aperture of lenses **614** and **613** matches the exit fiber optic **732**. The V is tilted with respect to the vertical plane to direct excitation light that is specularly reflected from the surface of the microwell **200** away from the light collecting leg of the V. Therefore, this arrangement introduces a spatial separation of emission and excitation light in addition to the spectral separation, and significantly improves the signal-to-noise ratio. This tilted V arrangement can also be used to conduct fluorescence polarization measurements.

The entry ports A and B of the excitation-emission separation device **600** accept fiber bundles from the excitation spectral device **500**. Fibers can be positioned to direct light that is spectrally separated by filters in the excitation spectral device **500** into input B of Block **620**. Fibers can also be positioned to direct light spectrally separated by monochromators in the excitation spectral device **500** into input A of Block **610**. Alternatively the inputs can be reconfigured by switching fibers **522** and **532**. This switching may be accomplished manually. The emission light is gathered by fibers **731** and **732** from ports C and D. The placement of fibers **731** and **732** in the exit ports C and D determines the origin of the emission light in the fibers.

FIG. **7** illustrates the holder **627** with associated dichroic mirrors **623**, **628**, and **629** and linear polarizers **624**, **625**, and **626** according to an exemplary embodiment of the present invention. The holder **627** is affixed to the slider **650**, which slides along rail **651** due to the applied force from the motor **652** through the belt **653**. The holder **627** moves in a direction perpendicular to the plane defined by the optical axes of the excitation and emission light. Although two different fibers **522** and **532** could occupy the fiber position depicted in FIG. **7**, for the sake of clarity only fiber **522** is shown.

In the depicted design there are five possible positions for the holder **627** relative to the fiber **522**, which delivers the excitation light. The first position, which is depicted in FIG. **7**, represents a situation where the center of mirror **628** is aligned with the optical axis of the fiber **522**. In this position fluorescence polarization based assays cannot be conducted. If the holder **627** is moved to the left for a distance equal to the distance between the centers of mirror **628** and **629**, the holder **627** will be in the second position. In the second position, the mirror **629** plays an active role, and fluorescence polarization based assays cannot be conducted.

The three other positions of the holder **627** correspond to three different situations. First, when the right third of the mirror **623** is positioned in front of the fiber **522**, fluorescence polarization based assays cannot be conducted. Second, when the middle third of the mirror **623** is positioned in front of the fiber **522**, the linear polarizer **624** is in the optical path of the excitation light, and the linear polarizer **626** is in the optical path of the emission light. In this case the polarization vectors of the excitation and emission light are crossed. Third, when the left third of the mirror **623** is positioned in front of the fiber **522**, the linear polarizer **624** is still in the optical path of excitation light, and another linear polarizer **625** is in the optical path of the emission light. In this case the polarization vectors of the excitation and emission light are parallel. Thus the linear motion of the holder **627** not only selects which mirror is placed in the optical path, but also allows for fluorescence polarization measurements.

As shown in FIG. **7**, the linear polarizers **625** and **626** have parallel surface orientations and perpendicular polarization axis orientations. They have active areas of equal sizes, and each size is comparable to the size of the cross-section of the emission light. The polarization axis of the linear polarizer **624** is parallel to the polarization axis of the linear polarizer **625**, and perpendicular to the polarization axis of the linear polarizer **626**. The area of the linear polarizer **624** is at least twice the area of the linear polarizer **625**. The area of the mirror **623** is at least three time the area of the linear polarizer **625**. The mirror **623** is partially reflective and partially transparent.

FIG. **8** shows a view from above the microplate **300**, along vertical axes toward the microplate **300** of the block **610** of the excitation-emission separation device **600**. Points A and B' are input portals of the excitation-emission separation device **600**. Lenses **611**, **612**, **663**, and **664** focus excitation light onto the microwell **200** in the microplate **300**. Lenses **613**, **614**, **673**, and **674** collect emission light and focus it into points C and D', which are exit portals of the excitation-emission separation device **600**. Standard **384** well microplates have an upper edge with a nearly square shape. The optical axes of lenses **611**, **612**, **663**, **664**, **613**, **614**, **673**, and **674** are oriented along the diagonals of microwells **200**. Using this arrangement a reading may be taken on the same microwell **200** simultaneously via filter-based or monochromator-based spectral systems. Because the excitation light from point A is reflected toward point B' and vice versa, very little excitation light is reflected toward exit portals C and D'. Therefore, the emission light is spatially separated from the excitation light.

FIG. **9** illustrates the structure of the emission spectral device **700** according to an exemplary embodiment of the present invention. In a preferred embodiment, the emission spectral device **700** has two spectral selection devices, which differ by the physical technology by which they separate light with different wavelengths. The first device is a filter selection device **710**, which has a variety of filters **711**. The second device is a double monochromator **720**. As shown in FIGS. **6** and **9**, fibers **731**, **732**, and **735** extend from their respective locations within the excitation-emission separation device **600** into the emission spectral device **700**. The selector switch **760** is used to direct light from fibers to the filter selection device **710**. FIG. **9** shows that the fiber **731** from fluorescent measurement block **620** and the fiber **735** from the luminescence measurement block **630** are connected to the selector switch **760**. FIG. **9** also shows that the fiber **732** from the fluorescent measurement block **610** is connected to the monochromator **720**. However, the arrangement in FIG. **9** is merely exemplary, and a user can change the connections by physically switching the fiber connections within the excitation-emission separation device **600**, or within the emission spectral device **700**.

In a preferred embodiment, the exit portals of the excitation-emission separation device **600** are in close proximity to the input portal **730** of the emission spectral device **700**. Points E and F may represent the exit portals of the emission spectral device **700**. The detector **800** may comprise two photomultiplier tubes (PMTs) positioned at points E and F (not shown).

FIG. **10** shows a fluid dispenser **1100** according to an exemplary embodiment of the present invention. The purpose of the fluid dispenser **1100** is to inject fluid into microwells **200** to initiate the reaction under investigation. Often the time from initiation to the time measurements have to take place is very short. Therefore, the injection ports **1101** and **1102** may be placed in close proximity to the optical reading system. Further, two separate fluid lines **1111** and **1112** may be used. Each fluid line connects to the stepper motor driven syringe drive for positive displacement fluid delivery. A three-way valve alternately connects a syringe to supply bottles on a suction stroke or to an injector line for dispensing.

In view of demand for investigating live cells, the multi-detection systems discussed below may further include the ability to image contents of the samples, along with obtaining quantitative data by fluorescence, absorbance, or luminescence, and the ability to provide in the same multi-detection analyzer a controlled gas atmosphere for samples preserving the long term viability of live cells.

FIG. **11**A illustrates a UMS according to an exemplary embodiment, in which an imaging subsystem is included in the UMS.

The UMS illustrated in FIG. **11**A includes a measurement chamber **320**, an excitation-emission separation device **600**, an optional dispenser **1100**, and an imaging subsystem module **1200**. Samples are dispensed into the array of microwells **200** in the microplate **300**. The microplate **300** is transported by the carriage **310** into the measurement chamber **320**, which may be incubated, and is positioned sequentially for measurements. The microplate **300** may be a matrix-styled receptacle for holding slides or wells of sample.

The imaging subsystem module **1200** has visual access to the microwells **200** of the micro plate **300** located on the carriage **310** housed in the measurement chamber **320**. Further details of the imaging subsystem module are discussed later below with respect to FIGS. **12** and **13**.

The imaging subsystem module **1200** includes an independent light source **1201** such as, for example a full spectrum or single color light-emitting diode (LED), that sends light via an epi-fluorescence Excitation/Emission filter cube **1210** and mirror **1220** into a microscope objective **1230**.

FIG. **15** illustrates a turret having objectives of the imaging module, according to an exemplary embodiment.

The microscope objective **1230** may be disposed on a turret **1240**, which rotates and/or changes position to change objectives and moves up and down to focus the microscope objective **1230** onto the microwells **200**.

By moving the turret with objectives vertically relative to microplate **300**, the focusing of the objectives onto the object of interest can be accomplished. The same vertical motion allows for the entry of the objective into incubation chamber **320** and removal of the objective out of the chamber when the imaging system is not in use.

As illustrated in FIG. **15**, the rotating objective turret may be mounted on motorized vertical linear way that allows insertion of one of the objectives at a time into incubation chamber and allow for focusing of the objective on the sample. A thermal barrier plug **1231** may be installed in addition to an objective **1230**, and may be used to close the incubation chamber when imager is not being used for sample observations. Accordingly, maximized sample chamber temperature uniformity may be obtained when imaging modality is in use or is not being used.

The image of the microwell **200** is imaged by tube lens **1250** and a camera **1260**.

FIG. **14** illustrates the design and positioning of the LED illumination module **1201** and the filter cube **1210**. The lower LED module **1201** may include an LED and a focusing lens. The filter cube **1210** includes an excitation filter **1211**, a dichroic mirror or partial mirror **1212**, and an emission filter **1213**.

The coordination of elements of the imaging subsystem module **1200** and motions of the microplate are controlled by a controller, which may be separately embodied or combined with a controller **1000** of FIG. **2** that also controls operation of the other multi-detection reading modalities. The controller may be a processor (e.g., central processing unit, microprocessor) that executes instructions stored in a memory for performing the imaging. The imaging of the microwell **200** contents can thus can be interlaced as part of an assay (steps in multimode plate processing discussed above). As a result, the samples that have undergone processing may be imaged.

Implementation of the imaging subsystem module **1200** is not limited to the configuration illustrated in FIG. **11**A and FIG. **13** as discussed below. For example, the UMS **100** of FIG. **2** may be modified to further include the imaging subsystem module **1200**. Similarly, the imaging subsystem module **1200** may be incorporated into the various embodiments of FIGS. **2** to **10** discussed throughout the application.

FIG. **11**B illustrates a UMS according to an exemplary embodiment, in which an imaging subsystem is included in the UMS.

The UMS illustrated in FIG. **19** includes a measurement chamber **320**, a monochromator based measuring system **12036** and filter based measuring system **12035**, an optional dispenser **1100**, and an imaging subsystem module **1200**.

As illustrated in FIG. **11**B, the measurement chamber **320**, the monochromator based measuring system **12036**, the filter based measuring system **12035**, the optional dispenser **1100**, and the imaging subsystem module **1200** are separate systems that can be independently controlled and manipulated.

Though the systems of FIG. **11**B may be independently controlled, the systems may be implemented in a common housing, as will be discussed below. Further, the systems may be controlled independently through a common interface, as will also be discussed below.

Samples are dispensed into the array of microwells **200** in the microplate **300**. The microplate **300** is transported by the carriage **310** into the measurement chamber **320**, which may be incubated, and is positioned sequentially for measurements. The microplate **300** may be a matrix-styled receptacle for holding slides or wells of sample.

Monochromator based measuring system **12036** could be used for fluorescence measurements, chemiluminescence measurements and absorbance measurements. The monochromator based measuring system **12036** may include a broad band light source **13001**, double excitation monochromator **13002** (stage **1**) and **13003** (stage **2**). The excitation light is delivered to sample via fiber optics **13005** and focusing lens **13020**. The emission light is picked up by focusing lens **13020** and via fiber bundle **13005** is guided to the double emission monochromator **13010** (stage **1**) and **1311** (stage **2**), and then to detector **13021**. The emission path may also be used for chemiluminescence measurements. The excitation double monochromator may direct light via fiber **13030** to the absorbance measuring lenses **13040** and **13050** and onto detector **13060**. The well **200** is positioned under the light beam for a specific measurement.

The filter based measuring system **12035** may be used for fluorescence measurements, chemiluminescence measurements and absorbance measurements. The filter based measuring system **12035** may include light source **14001**, excitation/emission cube **14010**, focusing lens **14020**, and detector **14021**. In case of absorbance measurements a lens **14040** focuses radiation that passed through the microwell **200** onto detector **14050**.

The imaging subsystem module **1200** has visual access to the microwells **200** of the micro plate **300** located on the carriage **310** housed in the measurement chamber **320**. Further details of the UMS including the imaging subsystem module are now discussed below with respect to FIGS. **12** and **13**.

FIGS. **12** and **13** illustrate a UMS according to an exemplary embodiment, in which atmospheric control is implemented.

In view of interest on the part of researches to work with live cells and perform long kinetic studies, while obtaining both quantitative (i.e., fluorescence, absorbance, luminescence, etc.) information and qualitative information (i.e., imaging, etc.), it is important to provide an environment for sample that is conducive to the cell's long term viability. This may be accomplished by controlling gas atmosphere around the cell samples. As will be discussed below, the multi-detection analyzer of FIG. **12** allows combined quantitative modality, such as filter or monochromator based optical measurements, along with quantitative imaging of samples, and the ability to conduct experiments and obtain measurements in a controlled gas environment.

The UMS **12001** includes a single housing **12017** and a dual-purpose base structure, which includes a base **12100** and a rear plate **12120**. The single housing **12017** creates one common atmosphere within the UMS. The single housing **12017** is designed to be substantially, and preferably completely, gas tight. The plate **12100** supports elements of the equipment compartments and sample compartments, but the plate **12100** does not separate the atmosphere of the elements of the equipment compartments and the sample compartments. That is to say, the elements of the equipment compartments and the sample compartments are subjected to the common atmosphere created by the housing **12017**.

The sample compartment may include a micro plate **12004**, similar to the microplate **300** discussed above, on carrier **12005** that is surrounded by top incubator plate **12105** and bottom incubator plate **12110**, and may not be sealed by the top incubator plate **12105** and the bottom incubator plate **12110** when inside the housing **12017**. The microplate **12004** may include microwells in which samples are contained.

The UMS **1201** includes a filter detection module **12035** and a monochromator detection module **12036**. Both the filter detection module **12035** and the monochromator detection module **12036** may contain motors and light sources, which generate heat when operated. As testing on the samples may require an ambient temperature, the heat generated by the filter detection module **12035** and the monochromator detection module **12036** should be effectively removed from the inside of the instrument housing **12017** to maintain temperature in the sample chamber close to ambient, in particular when incubation of the sample is not required. Preferably, the generated heat may be removed without introduction of air inside the instrument and discharge of the introduced air for cooling. Particularly, as the introduction of cooling air may carry dust particulates, which may increase sample evaporation and introduce errors in sample imaging and analysis.

The heat generated by the filter detection module **12035** and the monochromator detection module **12036** in the equipment compartment is conductively channeled via base **12100** to the rear plate **12120**. Heat transferred through the base **12100** to the rear plate may be removed by forced convection by a fan **12130** that is external to the housing **12017**. Thus, there is no need to introduce cooling air, the flow of which and the potential contamination by which makes incubation of samples difficult, inside the housing **12017**.

As a result, temperature within the housing **12017**, and thus temperature of the sample compartment may be controlled.

The base of the imaging module **1200** may be attached to the same base plate that holds filter module **12035**, monochromator module **12036**, and microplate transmission module **310**.

In addition to temperature control, composition of the atmosphere may also be controlled by a gas controller module, an overall view of which is illustrated in FIG. **16**.

Referring to FIGS. **12** and **13**, gas introduced from gas module **12006** via line **12038** and injector **12021** is dispersed via fan **12022**. The lines **12038** may also include a sampling gas line coming to gas module **12006**, which regulates the gas mixture delivered to the system by injector **12021**. The gas module may be separately embodied or combined with a controller **1000** that may also control operation of the other multi-detection reading modalities. The introduced gas fills the single housing **12017**. Accordingly, the elements of the equipment compartments and sample compartments are subjected to the introduced gas in the atmosphere within the housing **12017**.

Unlike cooling air, which is conventionally drawn into the chamber to cool equipment, the introduced gas may be initially drawn into the chamber. Once the atmosphere of the housing **12017** is sufficiently stabilized, flow of the introduced gas may be terminated. Accordingly, sample incubation and measurement may then take place in a stable environment, in which both temperature and atmospheric concentration are precisely controlled.

Implementation of housing **12017**, dual-purpose base structure, fans, and other elements for controlling temperature of the UMS **12001** and temperature and composition of the atmosphere within the housing **12017** is not limited to the configuration illustrated in FIGS. **12** and **13**. Rather, for example, the UMS **100** of FIG. **2** may be modified to further include such temperature and atmosphere control elements. Similarly, the temperature and atmosphere control elements may be incorporated into the various embodiments of FIGS. **2** to **11** discussed throughout the application.

The housing **12017** illustrated in FIGS. **12** and **13** may be a clam shell design, consisting of two half enclosures that are mated together around the main mechanical assembly. The main mechanical assembly by be suspended in the enclosed housing **12017** when both halves of the clam shell are mated together. To ensure that the completed housing **12017** is essentially gas tight, for example, a gasketing material could be used on the interface between the two halves. The use of the gasketing material is not limiting, as other forms of sealing may be employed.

The controlled environment of FIGS. **12** and **13** enables controlled ambient conditions, such as temperature and/or atmospheric concentration (e.g., CO_2 and O_2 concentration) around the samples. When multiple measurement modalities including imaging studies are required, all dedicated instruments should to be placed into environmental chamber, which creates problems with access and a need for manual intervention during a long term experiment. However, the controlled environment of FIG. **12** permits easy long term, multifaceted experiments to be conducted.

As discussed above, exemplary embodiments of the present invention provide a Universal Multi-detection System for determining fluorescence, chemiluminescence, and/or light absorbance of a sample in a microplate that allows the user to reconfigure and optimize the measurement system for a particular modality. The Universal Multi-detection System allows the user to choose the best measurement method for his assay. The user can select interference filters for their low detection limits and ability to run FP, TRF, and HTRF measurements with state of the art results, dual monochromators for their wavelength flexibility and spectral scanning, or a combination of both filters and monochromators. Both the excitation spectral device **500** and the emission spectral device **700** may contain interchangeable filter systems and monochromators.

In addition to providing unmatched flexibility, the Universal Multi-detection System also opens an avenue to run experiments that were not previously possible. For example, in the fluorescence mode, when only a small amount of an unknown fluorofore is available and it is not possible to increase the signal in the monochromator-based system by increasing the sample concentration, the user can obtain rough excitation and emission scans by using monochromators **530** and **720** in the excitation spectral device **500** and the emission spectral device **700**, respectively. Based on these initial excitation and emission scans, the user can then select an appropriate filter **521** to excite the sample with light that causes much stronger emission from the sample. The stronger emission spectrum can then be re-recorded. The user can also select a filter **711** to replace the emission monochromator **720** and re-record the excitation spectrum. It is important to have high-quality measurements of both the excitation spectrum and the emission spectrum. This process increases the emission signal and the signal-to-noise ratio, resulting in improved excitation and emission spectra, as compared with spectra obtained with just a monochromator-based system. It also allows a user to work with an unknown sample, and optimize the measurement conditions for that sample.

This approach can also be used to achieve maximum sensitivity in end-point reads. The user can select particular excitation wavelengths by using a monochromator **530** in the excitation spectral device **500**, and transmit the emission light through a filter **711** in the emission spectral device **700**. A similar benefit can be obtained by using a filter **521** in the excitation spectral device **500** with a monochromator **720** in the emission spectral device **700** during an end-point read. These methods are particularly suited to enhancing performance of environmentally sensitive labels, where variations in conditions give rise to perturbations of excitation or emission spectra, such as ion sensitive probes, pH sensitive probes, spectral shifts in polar dyes with conjugation, and binding and membrane probes.

FIG. **17** is a functional block diagram that illustrates the control of modalities of a UMS, according to an exemplary embodiment.

As illustrated in FIG. **17**, operation of the modalities of the UMS may be controlled by a central control unit (e.g., processor, CPU, microprocessor, etc.). The processor may be connected to communicate with and control elements of the sample environment, elements of sample selection and positioning, elements of the monochromator module, elements of the filter module, and elements of the imager module.

Elements of the sample environment under control may provide temperature control and gas control, as discussed above.

Sample positioning may be controlled through the use of motors for positioning samples in any of X and Y directions.

Elements of the monochromator module under control may include monochromator excitation, monochromator emission, monochromator PMT, and a light source such as a flash lamp.

Elements of the filter module under control may include the filter selector, a filter PMT, and a light source such as a flash lamp.

Elements of the imager module under control may include an objective selector, an image capturing device such as a camera, a focus drive imager, and an LED selector and control.

FIG. **18** is a flowchart of control method of a UMS, according to an exemplary embodiment.

Control of the UMS may be coordinated through use of the processor, as discussed above with respect to FIG. **17**. Input to the UMS (step S**1805**) may be accomplished through a local user interface of the UMS, such as a touch pad, or through communication with the UMS over a wired or wireless connection, such as over a network.

In the case of input to the UMS, input may be performed through the use of a user interface or graphical user interface displayed on a computer or other terminal that executes a control application.

The input may be user input, such as setting and parameters for executing control of the UMS.

In response to receiving input, control of the UMS may be effectuated through the various elements of the UMS, discussed above regarding FIG. **17**. For example, in response to receiving user input, the UMS may be controlled to execute a gas control procedure of the gas module (step S**1810**), a sample positioning control procedure to control positioning of samples (step S**1820**), a monochromator and/or filter control procedure to control operations of the monochromator and/or filter (step S**1830**), an imager control procedure to control the imager (step S**1840**), and to output a result of the controlling of the elements of the UMS (step S**1850**).

Although control is presented as illustrated in FIG. **18**, elements may be individually controlled in any sequence, and control of all elements is not required. Accordingly, the multiple modalities of the UMS may be controlled in a single assay. Additional aspects of the control of the UMS will be discussed below with respect to FIGS. **19** to **29**.

The control method illustrated in FIG. **18** may be implemented through execution of a processing unit (e.g., CPU) controlling elements of the UMS by executing one or more control programs. The programs may be stored in a memory (i.e., RAM, ROM, flash, etc.), or other computer-readable medium (i.e., CD-ROM, disk, etc.). The program may be executed locally by the UMS, or by a control apparatus, such as a computer that transmits commands to be executed by the UMS.

FIG. **19** illustrates a control apparatus for controlling a UMS, according to an exemplary embodiment.

As discussed above with respect to FIGS. **17** and **18**, the UMS may be controlled through communication with a control apparatus. The control apparatus may be a laptop computer, as illustrated in FIG. **19**. Communication between the UMS and the control apparatus may be conducted locally through a USB cable, as illustrated in FIG. **19**.

The control apparatus is not limited to the laptop computer, but may be any apparatus including a processor that executes control software for providing a user interface (UI) for controlling operations of the UMS. The control software may be installed on the control apparatus, or executed by the control apparatus in communication with a server device that hosts the control software, for example over a network such as the Internet.

The control apparatus executing the control software may issue commands to the UMS over the USB connection, though communication may be performed using other wired techniques, such as Ethernet, or wireless techniques such as infrared or wireless communication. The communication may be direct, for example over the USB connection, or accomplished through a network of intermediary devices, such as routers and switches. The network may be a local network, such as a local area network (LAN), or over a public network, such as the Internet.

FIG. **20** illustrates operations of an imaging subsystem of a UMS, according to an exemplary embodiment.

As discussed above, the UMS may include an imaging subsystem for imaging a sample.

The control software may include a UI for the imaging subsystem. The UI for the imaging subsystem may receive user inputs for controlling the imaging subsystem to image a sample.

As illustrated in FIG. **20**, the UI for the imaging subsystem may control various aspects of the imaging subsystem including, but not limited to, parameters such as color, lamp power, and lens magnification.

The UI for the imaging subsystem may further select at least one well at a position of a microplate and a position of imaging a particular portion the well. As illustrated in FIG. **19**, the imaging subsystem may control the UMS to image individual samples one at a time, but may also be controlled to image an entire microplate.

Other options of the UI for the imaging subsystem may be used to control focus, signal, and digital zoom.

The settings of the imaging subsystem may be stored in memory, and retrieved for subsequent imaging of additional samples.

The UI for the imaging subsystem may be employed to view live samples in real-time, review images of previous samples, and view reports of sample images.

As illustrated in FIG. **19**, the imaging subsystem may control the UMS to image individual samples one at a time, but may also be controlled to image an entire microplate.

FIG. **21** illustrates mode selection of operations of a UMS, according to an exemplary embodiment.

As discussed above, the UMS may include an analysis subsystem for analyzing a sample and an imaging subsystem for imaging the sample.

FIG. **21** illustrates the configuration in which the imaging by the imaging subsystem is selected. Naturally, other modes of the analysis subsystem, such as absorbance, luminescence, or the like, may be selected.

When selecting an analysis mode, the optics may also be selected by the user, such as monochromator or filter. Alternatively, the optics may be automatically selected based on the mode selection.

A read type may also be selected, such as endpoint/kinetic, spectral scanning, or area scanning Again, the read type may be selected by the user or automatically selected based on the mode selection.

FIG. **22** illustrates parameter settings of an imaging subsystem of a UMS, according to an exemplary embodiment.

Upon selection of a detection mode, additional parameters may be set.

In the case of selecting the imaging mode illustrated in FIG. **21**, the parameters for the selected imaging mode are shown in FIG. **22**.

The selectable parameters may include color, lens, focus, gain, and additional options, which are associated with the selected mode. An imaging speed and color for sample imaging may also be selected per well, or for the entire microplate.

FIG. **23** illustrates parameter settings of an analysis subsystem of a UMS, according to an exemplary embodiment.

As discussed above, the UMS may include an analysis subsystem for analyzing a sample and an imaging subsystem for imaging the sample.

FIG. **23** illustrates the configuration in which the analyzing by the analysis subsystem is selected. While the detection mode selected in FIG. **23** is the fluorescence intensity analysis, other modes of the analysis subsystem, such as absorbance, luminescence, or the like, may be selected.

When selecting an analysis mode, the optics may also be selected by the user, such as monochromator or filter. Alternatively, the optics may be automatically selected based on the mode selection. In the case of the fluorescence intensity analysis, the optics for luminescence fiber and imaging may be automatically deselected (e.g., grayed out).

FIG. **24** illustrates parameter settings of an analysis subsystem of a UMS, according to an exemplary embodiment.

Upon selection of a detection mode, additional parameters may be set.

In the case of selecting the fluorescence analysis mode illustrated in FIG. **23**, the parameters for the selected fluorescence mode are shown in FIG. **24**.

The selectable parameters may include excitation, emission, optics positioning, gain, and additional options, which are associated with the selected fluorescence analysis mode. A read speed, read height, and a wavelength for sample analysis may also be selected per well, or for the entire microplate.

FIG. **25** illustrates settings of multiple subsystems of a UMS, according to an exemplary embodiment.

As discussed above, in addition to the imaging and analysis subsystems, the UMS may also include a temperature subsystem and a gas control subsystem.

FIG. **25** illustrates a procedure in which an assay temperature is set to 37 degrees Celsius by control of the temperature control subsystem, a carbon dioxide level is set to 5% by control of the gas control subsystem, a reagent is automatically dispensed, then shaking happens for 10 seconds, a kinetic measurement is initiated to automatically follow fluorescence and image changes over a 10 hour time period every 15 minutes, according to controls of the imaging and analysis subsystems.

Additional controls may be provided for independently controlling the various subsystems. For example, control operations of the various subsystem operations may be changed, paused, stopped, resumed, or scheduled based on a delay or timer.

FIG. **26** illustrates monitoring operations subsystems of a UMS, according to an exemplary embodiment.

As illustrated in FIG. **26**, subsystems may be programmed by the user. In FIG. **26**, fluorescence signal is monitored automatically, and when a pre-set condition is met, imaging starts. Based on the pre-set condition programmed by the user, only wells of interest matching the condition may be imaged.

The programming conditions may be stored to memory, and recalled from memory for use with additional samples. Alternately, the programming conditions may be modified as needed, and stored as additional programming conditions.

Default programming conditions may be provided in a UMS, for selection by a user according to frequently used programs. Alternately, new programming conditions may be adopted according to input of the user.

FIG. **27** illustrates results of operations of an analysis subsystem of a UMS, according to an exemplary embodiment.

As discussed above, programming conditions may be input by the user and the programming conditions may be carried out by the UMS.

FIG. **27** illustrates results obtained from executing the programming conditions illustrated in FIG. **26**.

As illustrated in FIG. **27**, wells have been read using standard fluorescence measurement, wells with initial result above 22,000 are automatically imaged, and a thumbnail image is displayed for these wells, in combination with the fluorescence result.

Alternately, as opposed to each well, only those wells satisfying the pre-set conditions may be displayed, thereby increasing viewing efficiency.

The use of such a programmed process allows for faster reading of sample and limiting the size of the final data file since only the relevant wells are imaged.

FIG. **28** illustrates a detailed result of operations of an analysis subsystem of a UMS, according to an exemplary embodiment.

As discussed above, a programmed process may be executed by the UMS and results may be cumulatively displayed.

As illustrated in FIG. **28**, the results may be selectively displayed. For example, a "well zoom" may be displayed when clicking on one of the image thumbnails.

In addition to the zoom image of the well, other statistics, graphs, charts, and raw data of the well may be presented.

In addition to display of results, data reduction tools may be provided, as shown in FIG. **29**.

For example, "Image Analysis" tools may be provided for object counting, transfection efficiency, and total intensity. As illustrated in FIG. **29**, "Cell Counting" based on image analysis may be an exemplary tool.

Exemplary embodiments of the present invention have been described for illustrative purposes, and those skilled in the art will appreciate that various modifications, additions and substitutions are possible without departing from the scope and spirit of the invention as disclosed in the accompanying claims. Therefore, the scope of the present invention should be defined by the appended claims and their legal equivalents.

What is claimed is:

1. A device for analyzing one or more samples, comprising:

a receptacle support configured to support a microplate comprising a microplate well configured to hold a sample;

an incubation chamber within a housing configured to incubate the sample;

an imaging subsystem disposed substantially below the receptacle support and configured to image the sample on a cell level from below the sample, the imaging subsystem comprising an objective that is movably supported to be inserted into and removed from the incubation chamber;

a non-imaging analyzing subsystem that analyzes the sample on a well level, the non-imaging analyzing subsystem configured to provide a first measurement modality to measure an absorbance of the sample, a second measurement modality to measure fluorescence of the sample, and a third measurement modality to measure chemiluminescence of the sample;

a positioning subsystem configured to position the receptacle support to one or more positions within the housing for the non-imaging analyzing subsystem to analyze the sample and the imaging subsystem to image the sample; and

the housing that forms an exterior of the device and that encloses the receptacle support, the analyzing subsystem, the positioning subsystem, the incubation chamber, and the imaging subsystem,

wherein the receptacle support, the analyzing subsystem, the positioning subsystem, the incubation chamber, and the imaging subsystem are integrated within the housing.

2. The device of claim **1**, further comprising:

a gas control module configured to control a composition of atmosphere within the housing.

3. The device of claim **1**, wherein the microplate comprises a plurality of microplate wells arranged in a matrix.

4. The device of claim **1**, wherein the positioning subsystem is further configured to position the receptacle support based on an index that identifies positions of a plurality of wells of the microplate.

5. The device of claim **1**, wherein the objective comprises a plurality of objectives mounted on a turret, the plurality of objectives mounted on the turret to be selectable.

6. The device of claim **1**, wherein the imaging subsystem comprises an imaging light source and the non-imaging analyzing subsystem comprises at least one separate analyzing light source.

7. The device of claim **1**, further comprising a controller configured to control at least one of atmospheric temperature within the incubation chamber and gas composition within the housing.

8. The device of claim **1**, wherein (i) one of the imaging subsystem and the non-imaging analyzing subsystem and (ii) the receptacle support share a commonly controlled atmospheric composition within the housing.

9. The device of claim 1, wherein the imaging subsystem, the non-imaging analyzing subsystem, and the receptacle support share a commonly controlled atmospheric composition within the housing.

10. The device of claim 1, further comprising:

a temperature control subsystem configured to control a temperature of an atmosphere at least within the incubation chamber.

11. The device of claim 1, further comprising:

a processor configured to control operations of the non-imaging analyzing subsystem and the imaging subsystem.

12. The device of claim 1, further comprising a temperature control subsystem configured to control atmospheric temperature and a gas control subsystem configured to control atmospheric composition around the sample within the housing to provide an environment for the sample that is conducive to cell viability.

13. The device of claim 10, wherein the temperature within the housing of the device is maintained at ambient temperature by:

a plate that conducts heat from an atmosphere within the housing;

a fan outside the housing that cools the plate through forced convection.

14. The device of claim 11, further comprising:

a user interface configured to receive a user input to control the operations of the non-imaging analyzing subsystem and the imaging subsystem.

15. The device of claim 12, wherein the receptacle support, the positioning subsystem, the non-imaging analyzing subsystem, the imaging subsystem, the gas control subsystem, and the temperature control subsystem are integrated within the housing.

16. The device of claim 15, further comprising:

a processor configured to control operations of the non-imaging analyzing subsystem, the positioning subsystem, the imaging subsystem, the gas control subsystem, and the temperature control subsystem.

17. The device of claim 16, further comprising:

a user interface configured to receive a user input to control the operations of the non-imaging analyzing subsystem, the imaging subsystem, the positioning subsystem, the gas control subsystem, and the temperature control subsystem.

18. A device for analyzing one or more samples, comprising:

a receptacle support configured to support a microplate comprising a microplate well configured to hold a sample;

an incubated measurement chamber within a housing that encloses the receptacle support and configured to incubate the sample;

an imaging subsystem disposed substantially outside the incubated measurement chamber, the imaging subsystem comprising a plurality of objectives movably supported to be inserted into and removed from the incubated measurement chamber, the imaging subsystem configured to image the sample on the cell level from below the sample;

a non-imaging analyzing subsystem disposed substantially outside the incubated measurement chamber, the non-imaging analyzing subsystem configured to analyze the sample on the well level, and configured to provide a first measurement modality to measure absorbance of the sample, a second measurement modality to measure fluorescence of the sample, and a third measurement modality to measure chemiluminescence of the sample;

a common to both the imaging subsystem and the non-imaging analyzing subsystem positioning subsystem, configured to position the receptacle support within the incubated measurement chamber; and

the housing that forms an exterior of the device and that encloses the receptacle support, the incubated measurement chamber, the analyzing subsystem, the positioning subsystem and the imaging subsystem,

wherein the receptacle support, the incubated measurement chamber, the analyzing subsystem, the positioning subsystem, and the imaging subsystem are integrated within the housing.

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